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Preface

Although production of the Manila (asari) clam fishery accounts for only 0.4% of the total fisheries production in Japan on a weight basis and 0.5% on a monetary basis, asari is one of the most favored bivalves in the Japanese seafood market. It is also popular in other East Asian countries, as well as North America and Europe. However, the national production of asari has been drastically decreasing in Japan since the mid 1980's. There is a need to gather our knowledge to advance scientific researches and develop countermeasures for the production decline.

The First International Symposium on Manila (asari) Clam was organized by the Fisheries Research Agency and held in Yokohama, Japan in 2008, and the second symposium in Busan, Korea in 2012, co-organized by LTMER-Korea, POSTECH and Jeju National University. The third symposium was held in Tsu, Mie, Japan from June 1 to 2, 2015, followed by a workshop in Minamiise, Mie on June 3, co-organized by the Fisheries Research Agency, the Graduate School and Faculty of Bioresources, Mie University and the Mie Prefectural Government. The third symposium was the first time to invite participants from Europe, North America, Asia and Africa altogether; scientists, students and experts from eleven countries gathered for this symposium.

The present special issue of Bulletin of Japan Fisheries Research and Education Agency is the proceedings of the Third International Symposium on Manila (asari) Clam. The symposium was a venue to be inspired by new ideas and information from different parts of the world, which could help us consider future directions to promote asari researches, how and what support we should provide, and what we could suggest to the administrative sectors and fisheries associations.

I would like to express my sincere thanks to Mie University, the Mie Prefectural Government and other organizations for their cooperation to organize the symposium. I am also grateful to the editorial board and the contributors for publishing the proceedings.

Fuminari Ito
Executive Director
National Research and Development Agency,
Fisheries Research Agency

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June 1 - 2, 2015

The Third International Symposium on Manila (Asari) Clam

-International Collaboration for Manila (Asari) clam Studies-

Co-organized by
Fisheries Research Agency
Graduate School & Faculty of Bioresources, Mie University
Mie Prefectural Government

Programme

Symposium

June 1, 2015 (Monday)

Venue: Ust-Tsu, Tsu, Mie, Japan

Registration: 9 : 30-9 : 45

Welcome Remarks and Opening Addresses ····· 9 : 45-10 : 00
Fuminari Ito (Executive Director, Fisheries Research Agency)
Yoshihiro Komada (President, Mie University)
Eikei Suzuki (Governor, Mie Prefecture)

Outline of the Symposium ····· 10 : 00-10 : 10

Session I, Manila clam fisheries in Asian countries ····· 10 : 10-12 : 00

1. Stocks and fisheries of asari in Japan
   Mitsuharu Toba (Tokyo University of Marine Science and Technology, Japan)
2. Perkinsus (Lester and Davis 1981) infection in the Manila clam (*Ruditapes philippinarum*) in Korea; species identification, impacts and spatio-temporal distribution
   Kwang-Sik Choi (Jeju National University, Korea)
3. Development of Manila clam industry in China
   Fang Jianguang (Yellow Sea Fisheries Research Institute, China)

Lunch Break and Poster Session····· 12 : 00-14 : 00

Session II, Manila clam fisheries in Western countries····· 14 : 00-15 : 45

4. Asari clam in France: 1972 - 2015
   Xavier de Montaudouin, (University of Bordeaux, France)
5. Manila clam introduction in the Sacca di Goro Lagoon (Northern Italy): ecological and economic implications
   Marco Bartoli (Parma University, Italy)
6. Status of Manila clam, *Ruditapes philippinarum*, culture and fisheries on the West coast of North America

Brian C. Kingzett (Vancouver Island University, Canada)

Break ····· 15 : 45–16 : 00

Session III, Genetic diversity of Manila clam and fishery ground restoration
····· 16 : 00–17 : 10

7. Mapping the stranger: genetic diversity of Manila clam in European coastal lagoons
   Stefania Chiesa (University of Aveiro, Portugal)
8. An a-priori evaluation model for restoration of fisheries population of the Mania clam and a countermeasure example
   Hisami Kuwahara (Fisheries Research Agency, Japan)

General Discussion ····· 17 : 10–18 : 00

Field trip

June 2, 2015 (Tuesday)

Field trip to fishing ground, aquaculture site and spat collection site of the Manila clam in Matsusaka and Toba, Mie.

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Bull. Jap. Fish. Res. Edu. Agen. No. 42, 7－8, 2016

Outline of the third international symposium on Manila (asari) clam

Satoshi WATANABE*1 and Junya HIGANO*1

The Manila clam (*Ruditapes philippinarum*, commonly known as asari in Japanese) is an important bivalve species for fisheries and aquaculture in many parts of the world. Asari is indigenous to East Asian countries, such as China, Korea and Japan, and it was accidentally introduced to Washington State, the United States in Pacific oyster (*Crassostrea gigas*) spat shipments from Japan in 1900. It quickly became naturalized in the west coast of North America and is now found and cultured from northern California to southern British Columbia, Canada. In 1972, asari was introduced to France from Oregon State as a substitute for the native *Ruditapes decussatus*, whose abundance had declined in Europe due to overexploitation. Asari is now produced not only in its native countries but in the United States, Canada, France, Spain, Italy, England, Ireland, Portugal, Norway, Germany, Belgium, Tunisia, Morocco and Israel (FAO, http://www.fao.org/fishery/culturedspecies/Ruditapes_philippinarum/en).

Japan used to produce the largest amount of asari in the world; however, the production peaked in the mid 1980's and has continuously declined for almost thirty years. The national fishery production (inclusive of bottom aquaculture) of asari in Japan was 12,700 metric tons 2013, which was less than 10% of the peak value. Asari production in China, on the other hand, has increased tremendously for the last couple of decades, and it is reported to be over 3 million metric tons per year in recent years. In Korea, asari production is relatively stable fluctuating between 20,000 to 40,000 metric tons. Europe, especially Italy, has a high production of asari, exceeding Korea and Japan. France is facing a decline in asari production in recent years. Relatively constant production of asari is seen in Canada and the United States.

Government agencies and research institutions in Japan have proposed and taken various countermeasures against the declining asari fishery production, but have had limited success. The first international symposium on Manila (asari) clam was held in Yokohama, Japan in 2008. The scope of the symposium was stock enhancement and management of asari, and information about asari fishery and aquaculture was exchanged amongst international participants. Japanese participants were particularly interested in aquaculture and inexpensive seed production technologies from other countries since asari production in Japan mostly relied on the wild catch and bottom culture with transplanted wild spats. The introduction of pathogens and invasive species along with shipments of spats from foreign countries was also an issue at that time. The second symposium was held in Busan, Korea in 2012. Regional and global partnerships for asari studies was the symposium scope.

The third international symposium on Manila (asari) clam was held at Ust Plaza in Tsu, Mie, Japan on June 1 and 2, 2015, co-organized by the Fisheries Research Agency, Mie University and the Mie prefectural government. The aim of the symposium was to compare the status of asari fishery and aquaculture in different countries in an attempt to identify factors affecting production levels and discuss future directions of asari research to work out countermeasures for the restoration and maintenance of fishery populations.

There were 115 participants from 11 countries. The keynote speakers were invited from Canada, China, France, Italy, Korea, Portugal and Japan, and there were 47 poster presentations. Various aspects of asari biology, ecology, fisheries, aquaculture and

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socio-economics were introduced and discussed. Disease, overexploitation, water oligotrophication, environmental water hypoxia, siltation, as well as social conflicts were reported as problems regarding asari fishery production. Fishery management, environmental management, seed quality control and promotion of aquaculture production were some of the key points expressed during the discussions.

The symposium was followed by a workshop at the National Research Institute of Aquaculture, Fisheries Research Agency in Minamiise, Mie on June 3. Statuses of asari fishery and aquaculture were reported from Canada, China, France, Italy, Japan, Korea, Portugal and Turkey to search for topics of possible future collaborative research projects.

These proceedings are a compilation of short papers based on the keynote talks, abstracts for poster presentations and country reports given at the workshop.

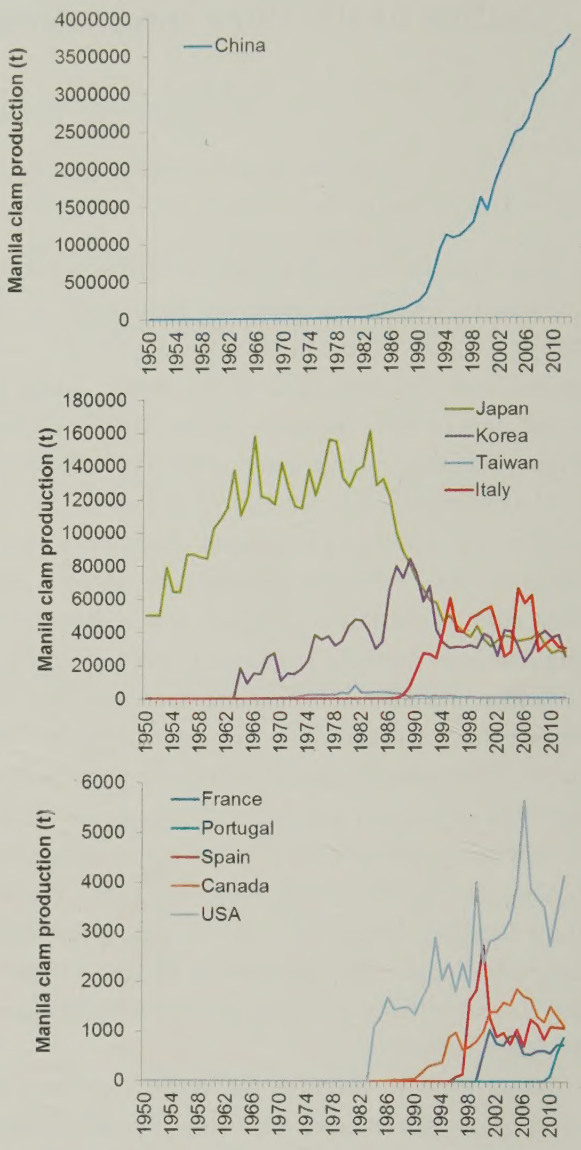

Fig. 1. Chronological transition of the annual Manila clam productions in major producing countries.

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Stocks and fisheries of asari in Japan

Mitsuharu TOBA*[1,2], Yutaka KOBAYASHI*[1,3], Jun KAKINO*[1,4], Hiroshi YAMAKAWA*[2], Ryo ISHII*[1,5], and Ryu OKAMOTO*[1]

Abstract: Asari clam is one of the most important fishery resources in Japan. The clam has been utilized as not only a food but also a recreational harvestable species. Main habitat of the asari extends from tidal area to shallow subtidal area of sandy soft bottom. Asari is harvested by hand raking or short-handled basket raking in tidal areas and long-handled basket raking, dredging, helmet diving in subtidal areas. Major areas of asari harvest are Tokyo Bay, Mikawa Bay, Ise Bay, Suo Nada and Ariake Bay. Annual harvest of the asari in the major areas, however, decreased largely after late 1980s except Mikawa Bay. Stock decline of natural asari is remarkable and seriously problematic for coastal fisheries in Japan. Along with application of the various countermeasures intended to restore the natural stocks of asari, a number of factors have been discussed to be responsible for the asari decline. Some of these are decrease of suitable habitat areas, deterioration of water and substrate quality, erosion of the clams by strong wave disturbance, new predators and parasites, oligotrophication, and excess harvesting. We still have not determined significant factors responsible for the asari decline in Japan. In the discussed factors, we compared the effect of five factors; blue tide, river flood, strong wave disturbance, infection of sea spider, and hypoxia on clam larvae, based on the studies in Tokyo Bay. The impacts (mortalities) of the factors for the total asari stock by the blue tide in Sanbanze in 2008, the river flood in Sanbanze in 2007, winter wave mortality in the three areas in 2009–2010, and infection of sea spider in Kisarazu in 2009 were calculated to be 0.16, 0.07, 0.95, 0.30, respectively. Among these factors, winter wave mortality showed the most significant effect on the asari stock in Tokyo Bay. Comparing the properties of the estimated factors, the factors that affected wider areas and longer period with higher mortality were supposed to give severer impact for the asari stock. In order to take effective countermeasures for the decline of asari stock in Japan, further assessment of the impact of the suspected factors is required to be progressed.

Key words: stock decline, causative factors, assessment, blue tide, winter wave mortality, sea spider

Asari is one of the most important fishery resources in Japan. Populations of asari are naturally maintained all over the coastal bay areas in the country. The clam has been utilized as not only a food but also a recreational harvestable species at least since eighth century AD. Asari is favored by common people because it inhabits abundantly in shallow sandy coast near urbanized areas and can be harvested easily as a palatable seafood.

Annual production of asari in Japan, however,

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declined during late 1980's and 1990's to ca. 40,000 t, after reaching its maximum of ca. 160,000 t in mid 1980's. Stock decline of natural asari is remarkable and seriously problematic for coastal fisheries in Japan.

A number of factors have been discussed to be responsible for the asari decline (National Conference on Asari Stocks, 2006). Some of these are decrease of suitable habitat areas by the land reclamation, deterioration of water and substrate quality due to coastal development, physical unstabilization of bottom substrate by strong wave disturbance, predators and parasites newly invaded during recent years, oligotrophication with strict regulation of waste water quality control, and excess harvesting.

There have been few discussions to discriminate the asari clam groups, distributed over Japanese Archipelago, into ecologically independent populations (stocks). Based on the reports on the spatial exchange of clam larvae among local clams within some bay areas (Hinata and Tomisu, 2005, Mizuno *et al.*, 2009, Tezuka *et al.*, 2008, Nishihama *et al.*, 2011), we assume the asari groups in the major harvested areas, Tokyo Bay, Mikawa Bay, Ise Bay, Suo Nada, Ariake Bay, as the independent asari stocks in this presentation. In this hypothesis, many of the discussed factors, which recognized to have apparent effects on the decrease of local clams in a bay area, have uncertain effects on the decrease of asari stocks in a whole bay area.

In order to design a restoration of the asari stocks, precise determination of causative factor(s) for the decline of asari stock is essentially important. Few studies, however, have been conducted to evaluate the quantitative effects of the factors for the decline of asari stocks.

In this presentation, we introduce firstly the present status of asari stocks and harvesting in Japan. Then we discuss the causative factors on the asari decline, presenting quantitative assessment of the impact of the factors which pointed to be responsible for the asari decline in Tokyo Bay, based on the recent results of field and laboratory studies.

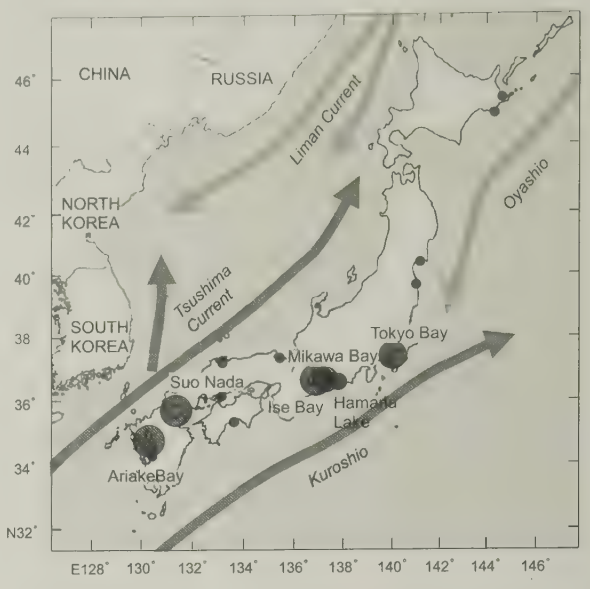

Fig. 1 Major (large shaded circles) and sub-major (small shaded circles) areas of asari clam harvest in Japan. Dark and light shaded arrows indicate warm and cold sea water current, respectively.

Harvest of Asari

Major areas and harvesting: Coastal areas in Japan can be divided into northern half and southern half based on the temperature of sea water currents flowing along the coastal areas (Fig. 1). Areas where the asari can be harvested are scattered all over the coastal areas in Japan. Major harvest areas locate in the southern half of Pacific coast; Tokyo Bay, Hamana Lake, Mikawa Bay, Ise Bay, Suo Nada and Ariake Bay. The reason why major areas locate in the southern area is not due to their marine environment but simply due to that there are many large bay and shallow areas. All of the clams being harvested in Japan are natural clams, except the clams harvested from some culture trials using artificial juveniles.

Clams are harvested by various methods; hand rake, basket rake, dredge and helmet diving (Fig. 2). Applied method depends mainly on the water depth of the place where harvesting is operated. Among these methods, short-handled basket rake ("Joren" or "Koshimaki"), which developed for the harvesting in tidal area, is used most frequently. Recreational harvest is important leisure industry in many areas.

* Statistical Yearbook of Production in Marine Fisheries and Aquaculture 1968–2010, Ministry of Agricultrue,Forestry and Fisheries.

Fig. 2 Methods for clam harvesting. Hand rake (a), Short-handled basket rake (b), long-handled basket rake (c), dredge (d), helmet diving (e) and recreational harvest (f).

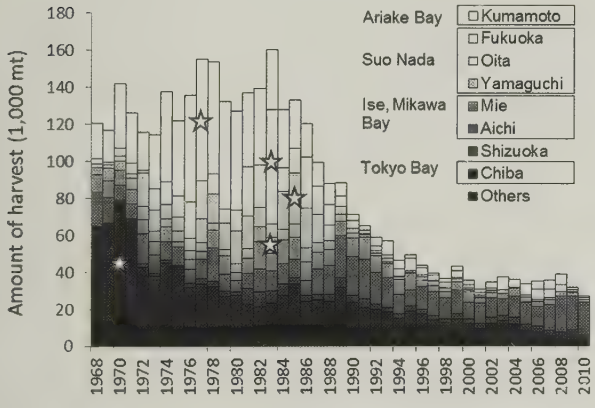

Fig. 3 Changes of the prefectural amount of asari harvest from 1968 to 2010. Star symbols indicate years of peak harvest in the major prefectures whose production decreased.

Production in the major areas: Asari harvest declined largely in the major prefectures after 1980s except Aichi and Shizuoka Prefecture (Fig. 3)*. The year of the peak production in Chiba prefecture (Tokyo Bay) is 1970. That of Kumamoto prefecture (Ariake Bay) is 1977, and Fukuoka, Oita and Yamaguchi prefecture (Suo Nada) is mid 1980s. Although the total production of the asari in Japan appears to have begun to decrease in late 1980s,

Fig. 4 Locations of the clam harvesting areas (black curves) in five major areas. Tokyo Bay (a), Mikawa and Ise Bay (b), Suo Nada (c), and Ariake Bay (d).

initiation and length of the periods of decrease in the major areas have time lags. The cause of the asari decline seems not to have affected simultaneously in the major prefectures.

Coastal area of Tokyo Bay has been highly developed for industry, commerce and residence (Fig. 4a). Most of the coastal areas in Tokyo Bay were wide tidal flat in early 1960s. In the present, shallow or tidal areas, where the clam can be harvested, remain in three separated places due to the land reclamation during 1970s–1980s. Some of the problems which depress asari production are common to the other major areas; *i.e.* mortality due to blue tide, river flood and strong wave disturbance in winter (Toba, 2002; 2004). Additionally, parasitic arthropod *Nymphonella tapetis* "sea spider" caused mass mortality in Kisarazu after, 2007 (Miyazaki *et al.*, 2010; Kobayashi and Toba, 2014).

Harvest of asari in Mikawa Bay has been maintained stable (Fig. 3b). Transplantation of juvenile clams from Rokujo tidal flat to the other coastal areas sustains substantially the clam harvest in Mikawa bay (Ishida *et al.*, 2005). Rokujo tidal flat, estuary of Toyokawa River, locates innermost of the bay and have an abundant natural spat fall every year (Kamohara *et al.*, 2014). Hypoxic water

often threatens survival of the clams particularly in subtidal zone during warm water season (Suzuki *et al.*, 2011).

Contrary to Mikawa Bay, natural spat fall in Ise Bay, which locates next to Mikawa Bay, has been decreasing (Mizuno *et al.*, 2009) (Fig. 4b). Juvenile clams settle mainly in the estuaries in southern part of Mie Prefecture. In addition to the spreading hypoxic water from northern urbanized and industrialized area to the southern bay, excess harvesting may have damaged the asari stock in Mie Prefecture after 1990s (Mizuno and Maruyama, 2009). Mortality of juveniles occurs more often in recent years caused by the river flood with increasing heavy rain (Mizuno *et al.*, 2009; Hanyu, 2015). Proliferation of asian mussel *Musculista senhousia* or sand dollar *Astriclypeus* sp., hampered growth and harvest of the clams considerably in spatially occupied subtidal areas.

Suo Nada locates easternmost of Seto Inland Sea and is surrounded by three prefectures (Fig. 4c). Harvest of the asari in Suo Nada, which exceeded 30,000 t in 1980s, decreased to near 0 t in 2010. Heavy harvesting effort using helmet diving and water injection dredge may have collapsed adult spawners of clam stock (Yamaguchi Prefecture, 2006; Ooita Prefecture, 2009). Heavy predation by eagle ray *Aetobatus flagellum* greatly depresses the asari production after 2000s (Fukuda and Zenitani, 2009). Migration of eagle ray extended to Seto Inland Sea with elevating water temperature. Additionally, oligotrophication, which is due to the strict regulation of waste water quality, is discussed to suppress organic production in the entire bay (Hamaguchi, 2011).

In Ariake Bay, as well as in other areas, asari harvest decreased in 1980s and did not recover (Fig. 3d). Strong harvesting effort (Nasu *et al.*, 2008) and spreading hypoxia (Fujii and Yamamoto, 2002) are pointed as the causative factor. River flood and strong wave disturbance in winter frequently causes mortality. Heavy infection of *Perkinsus olseni* is also discussed to be one of the causative factors (Hamaguchi *et al.*, 2002; Waki and Yoshinaga, 2013).

Alien species *Mercenaria*: Natural colonization of exotic clam species, ocean quahog *Mercenaria*

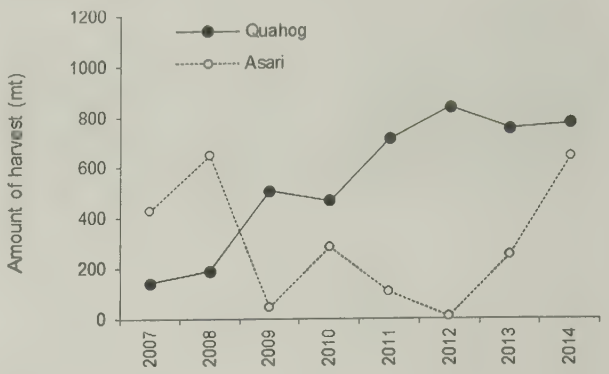

Fig. 5 Changes of the harvest of quahog and asari in Sanbanze, Tokyo Bay

mercenaria, was identified firstly in Sanbanze, Tokyo Bay, in 1998 (Hiwatari and Kohata, 2005). The quahog is considered to be introduced to Japan unintendedly with ballast water of ships from North America. Harvest of the quahog has been increasing to near 1,000 t in 2014 and it has become an important local fishery resource in Tokyo Bay (Fig. 5). The increasing harvest of quahog is contrasted to fluctuating harvest of asari. Quahog is reported to be able to tolerate low DO condition extended period by complete and sustained valve closure (Grizzle *et al.*, 2001). This tolerance may be able to survive quahog and increase its stock size in Sanbanze where native clams often suffer by hypoxic water. Quahog, however, is designated as invasive alien species in Japan. Although there is no legal restriction, artificial enhancement or culture of the quahog should be avoided.

Assessment of the Causative Factors of the Asari Decline

Factors to be assessed: After decrease of asari production became obvious in many areas in 1990s, numbers of the phenomenon were presented as possible responsible factors for the asari decline (Table 1). Presented factors can be grouped into five categories; coastal development, global warming, deterioration in water quality, immigration of troubled factors, and changes of fisheries activity. This table is the mixture of the factors which effect is apparent or not. For example, the land reclamation never fails to bring a clam decline through a

Table 1. Suspected factors responsible for the decline of manila clam.

Categories	Causative factor	Directly affected factor
Coastal development	Land reclamation	Decrease of suitable habitat area
	Perpendicular seawall	Changes of current and wave, decrease of current velocity
	River improvement	Decrease of sand supply to estuaries, increase of mud
	Heat island	High water temperature, short time heavy rain, strong wind
	Land subsiding	Decrease of suitable habitat area, intrusion of strong wave to shallow zone
Global warming	High temperature	High water temperature, changes of predators, food plankton and harmful plankton
	Extreme weather	Strong typhoon, heavy rain, bomb cyclone, river flood
	Elevation of sea level	Elevation of tidal level, intrusion of strong wave to shallow zone, change of current
	Climate change	High water temperature, increase of cloudy and rainy weather
Degradation of water quality	Eutrophication	Blue tide, hypoxia, red tide
	Pollution	Organotin, endocrine disrupter
	Excess regulation of waste water quality	Oligotrophication
Immigration	Disease and parasites	BRD, Perkinsosis, sea spider
	Predators	Eagle ray, new moon snail
	Transplantation	Genetic disturbance, competition
Changes in fisheries	Simplification of fisheries target species	Excessive harvesting effort

decrease of habitat areas. On the other hand, concerning on increase of mud and/or decrease of sand in the sediment due to river improvement, quantitative evidence which can evaluate its impact on a stock is not presented. As a result, we still have not reached a mutual understanding which factors are significant for the decline of asari stock. As an example of determining the significance of factors, we compare the effect of five factors; blue tide, river flood, strong wave disturbance, sea spider, and hypoxia on clam larvae, based on the studies in Tokyo Bay.

Tokyo Bay is surrounded by three prefectures; Chiba, Tokyo and Kanagawa (Fig. 4a). In these prefectures, harvest of asari in Chiba is overwhelming those of the other prefectures (Fig. 6). Therefore, we can tentatively assume the asari stock in Chiba as the asari stock in Tokyo Bay. The decline of the harvest in Chiba from 1960s to 1970s is apparently due to land reclamation. Asari harvest,

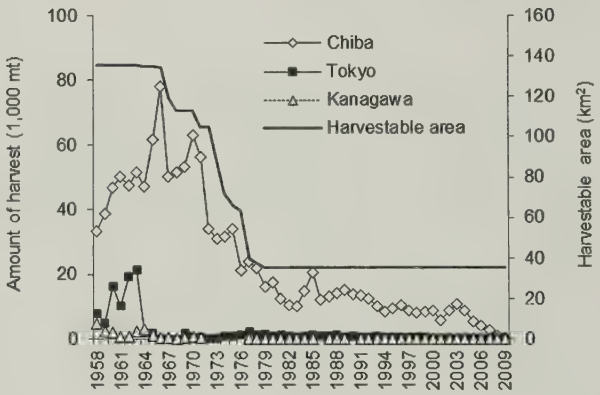

Fig. 6 Changes in the harvest of asari in three coastal prefectures in Tokyo Bay (Chiba, Tokyo and Kanagawa) and areas for the clam harvest in Chiba Prefecture.

however, decreased gradually and steadily after 1980s in spite of unchanged area of harvest in this period. In the followings, we examine the decrease after 1980s based on the clam density investigated

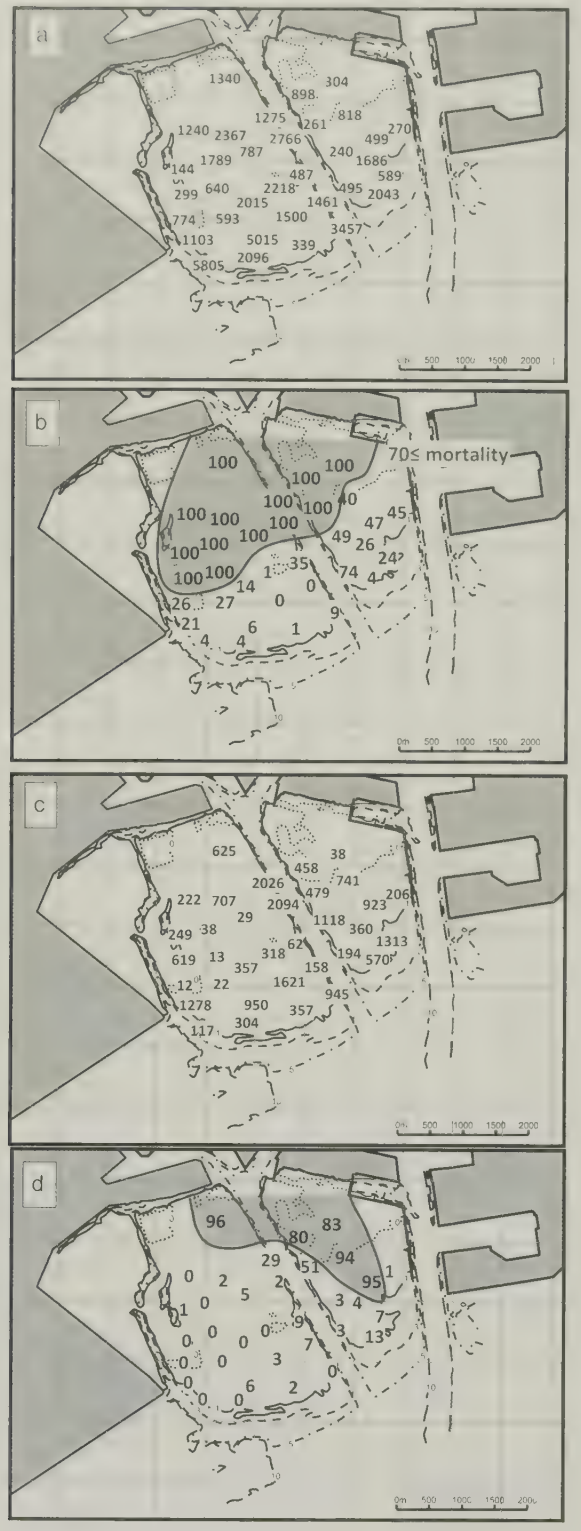

Fig. 7 Estimation of the impact of the mortality events in blue tide in 2008 (a, b) and river flood in 2007 (c, d) in Sanbanze. Figures a and c show the clam densities (inds m^{-2}) before the mortality events in the sampled sites, b and d show the calculated percent mortalities of the clams after the events. Shaded areas in b and d indicate mortalities higher than 70 %.

bimonthly in three areas in Chiba; Sanbanze, Kisarazu and Futtsu. In these areas, clam densities have been investigated using quantitative sampling gears and 2 mm square mesh sieve since 1980s.

Blue tide and river flood: Mass mortality of the clams due to blue tide occurred in Sanbanze in September 2008. Amount and rate of the mortality caused by blue tide could be estimated by the spatial density of the clams before and after the mortality event. Figs. 7a and 7b show the clam densities before the blue tide and calculated ratios of the mortality in sampled sites, respectively. Mean mortality and total amount of dead clams, which calculated from contour maps of previous density and mortality, were estimated to be 39% and 5,500 t, respectively. Likewise, mean mortality and amount of dead clams caused by the river flood in September 2007 were estimated to be 20% and 1,900 t, respectively (Figs. 7c, d).

Annual harvest and density of the clams in Sanbanze have been fluctuated and decreased since 1980s (Fig. 8a). In Sanbanze, mortality due to blue

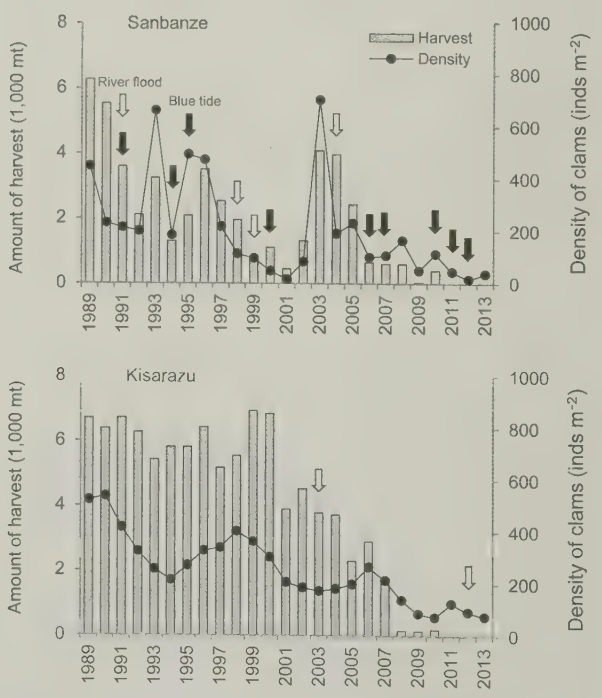

Fig. 8 Changes in the harvest and density of asari in Sanbanze and Kisarazu. Vacant and solid vertical arrows indicate the occurrence of the mortality due to river flood and blue tide, respectively.

tide or river flood occurred frequently in this period. In contrast, Kisarazu did not suffer blue tide during recent 25 years (Fig. 8b). The impact of blue tide and river flood differs largely with the areas. These influences have a marked locality in Tokyo Bay.

Winter wave mortality: Densities of the clams in Sanbanze and Kisarazu change regularly in every year with peak and bottom period in summer and winter, respectively (Figs. 9a, b). The ratio of the clam density in February to that in October in former year, which represents survival of the clams during winter (influence of harvesting was excluded), has been <0.1 every year in Sanbanze (Fig. 9c). In Kisarazu, survival during winter is decreasing to <0.6 in recent years (Fig. 9d). Winter mortality is the common phenomenon which gives significant impact on the asari stock in Sanbanze and Kisarazu.

Based on a number of investigations and experiments, we consider the cause of the winter mortality is erosion of the clams due to strong wave disturbance (Shibata *et al.*, 1997; Kakino, 2000). Clams sustained and remained under a cover net which spread over bottom surface of the tide land during winter, while ambient clams without cover net disappeared. Low temperature and poor nutritional condition in winter may depress the burrowing activity of the clams lower.

One of the significant driving factors for sediment transport is wave shear stress. In the field study in Kisarazu, the positive relationship was found between significant wave amplitude and velocity of wind in shoreward direction. Tidally changing water depth has also a relationship to the wave amplitude (Toba *et al.*, 2011; Toba and Kobayashi, 2011). The wave amplitude, which directly relates to the wave shear stress, is influenced by both strength of the wind and water depth. In the depth range of 0–2 m, increasing depth intensifies bottom shear stress with increasing intrusion of high wave from offshore to tidal area. Most of the harvestable area in Sanbanze is subtidal (±0–−2 m in mean low water level in spring tide) mainly because of the land subsiding due to intensive pumping up of underground water during the period of coastal development in 1960s–1970s.

Maximum wind velocity, which monitored at the meteorological observatory in Chiba City from 1964 to 2010, has not changed markedly in these 40 years (Fig. 10a). On the other hand, the changes of the

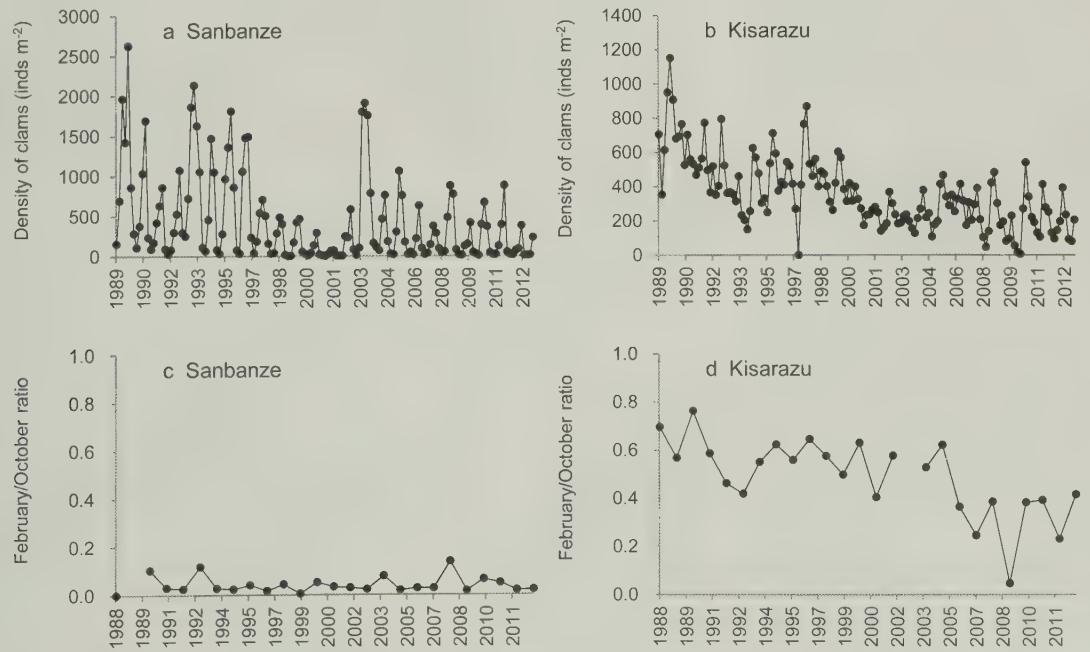

Fig. 9 Seasonal changes of the density of asari in Sanbanze (a) and Kisarazu (b), and ratios of densities in February to those in October in former year in Sanbanze (c) and Kisarazu (d).

mean tidal height, which measured at four of the automatic tide-gauge stations in the coast of Tokyo Bay from 1968 to 2008, obviously show the elevation tidal level during these 40 years (Fig. 10b). The increasing erosion of the clams in winter in Kisarazu may be caused by elevating tidal height.

Sea spider: Sudden mortality, caused by heavy infection of sea spider, occurred in Kisarazu in 2007 (Kobayashi and Toba, 2014) (Fig. 11). There had been few reports on the sea spider in last 90 years so that we had very little information on its biology. The route of infection and the reason of sudden outbreak are still not known. Infected clams are sucked body fluid by the sea spider. In our study, the prevalence of sea spider infection had a peak period in summer. Mortality of intensively infected clams occurred in this peak period.

The ratio of intensively infected clams (>5 sea spiders/clam) has been decreasing since first outbreak in 2007 (Fig. 12a). Comparing the spatial clam density before (2005–2006) and after (2007–2012) the outbreak of sea spider infection in Kisarazu, apparent decrease is found in 2009 (Fig. 12b).

Hypoxia on clam larvae: Low DO condition was experimentally confirmed to lower not only the survival but also the swimming activity of the clam larvae (Toba *et al.*, 2008). The larvae which encounter low DO water in natural environment may cease swimming and sink to the deeper layer of poorer condition of DO. And the vertical swimming position of the clam larvae was found to shift from

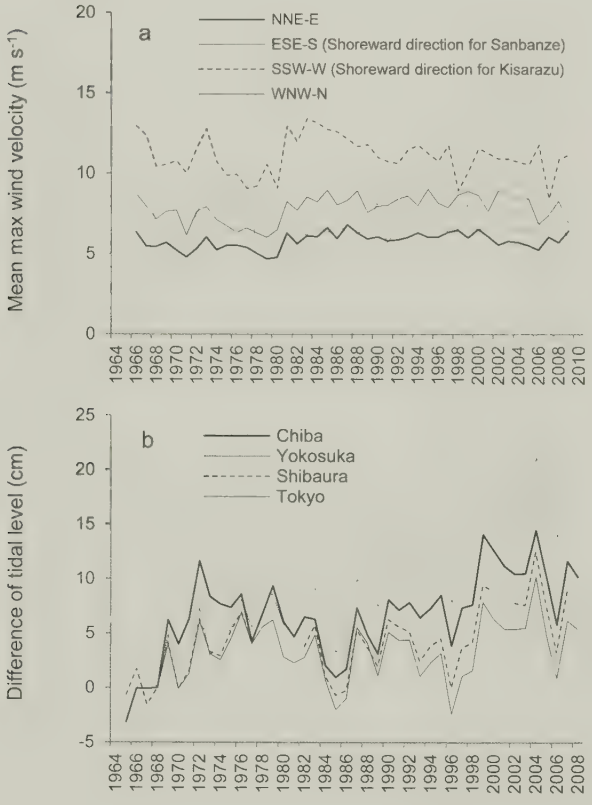

Fig. 10 Changes in the mean maximum wind velocity (a) and difference of mean tidal heights (b), calculated from hourly maximum wind velocity monitored at Chiba Meteorological Observatory and hourly tidal heights measured at four automatic tide-gauge stations in Tokyo Bay, respectively. Mean maximum wind velocity is divided into four directions. Tidal heights are the relative values as 1968 = 0 cm.

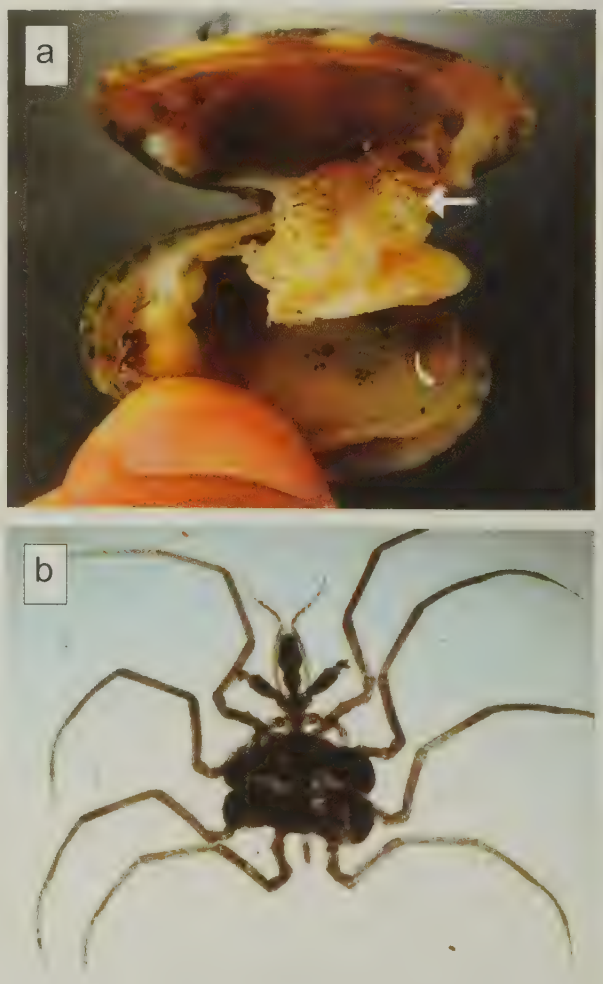

Fig. 11 Asari infected by larval sea spider (a, arrow) and free living adult of sea spider carrying egg sacks (b).

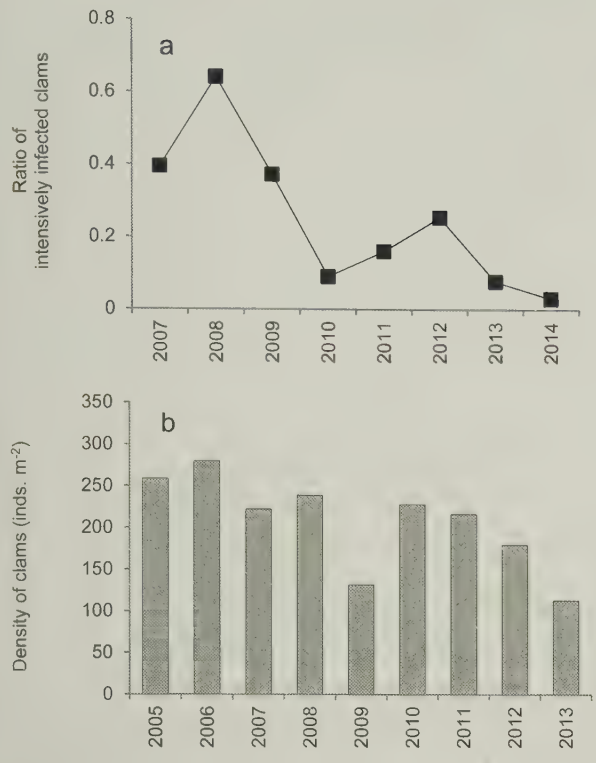

Fig. 12 Changes in the ratio of intensively infected clams (>5 sea spiders clam^{-1}) after outbreak of infection in 2007 (a) and spatial clam density in Kisarazu from 2005 (before outbreak) to 2013 (b).

surface to bottom layer with their growth even in normal DO condition (Toba *et al.*, 2012). Additionally, from May to October, low DO water is continuously formed in the bottom layer over wide extent in Tokyo Bay (Fig. 13) (Chiba Prefectural Fisheries Research Center, 2012). Moreover, the period when low DO water spreads over the bottom layer coincides with the period of spawning of adult clams in Tokyo Bay (Toba *et al.*, 1993; 2007). Those facts strongly imply that low DO water affects the larval survival in the natural environment in Tokyo Bay.

Comparison of the impacts of the factors: The impacts of the factors for the asari stock in Tokyo Bay were evaluated using total mortality calculated by decrease of number of clam individuals in the events in three local areas. Table 2a shows the calculation of the total mortality by the blue tide in Sanbanze in 2008. Total mortality was calculated by the clam densities before the event, areas of harvest, and local mortalities in the events in three areas. In this blue tide event in Sanbanze, mortalities in the other two areas were assumed to be 0. As a result,

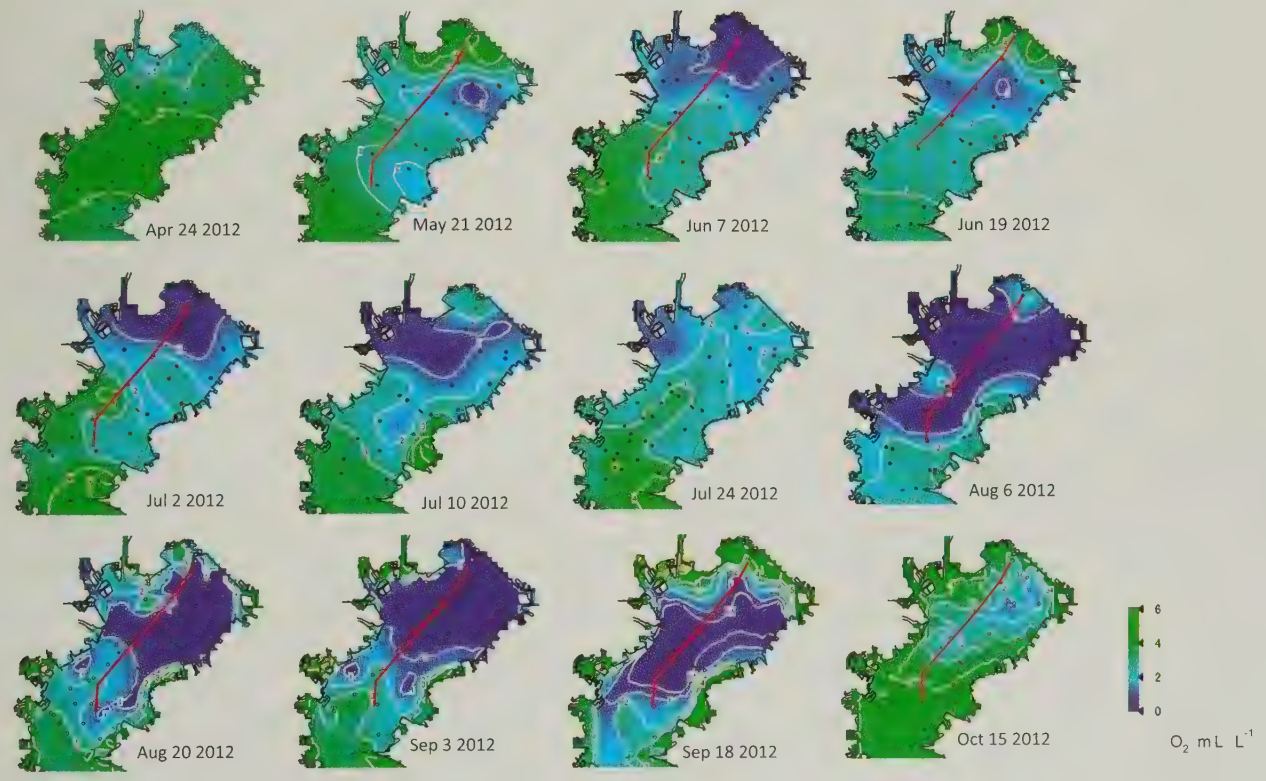

Fig. 13 Seasonal change of DO concentration in the bottom layer in 2012 in Tokyo Bay (Chiba Prefectural Fisheries Research Center).

the impact (mortality) by the blue tide in Sanbanze in 2008 was estimated to be 0.16 for the total clam stock in Tokyo Bay. In the same way, impact of the river flood in 2007, winter wave mortality during 2009–2010 and infection of sea spider in 2009 were calculated to be 0.08, 0.95 and 0.30, respectively

Table 2 Estimation of the impacts of the mortality events for the clam stock in Tokyo Bay.

a. Blue tide in Sanbanze in September 2008

Local area	Clam density before the mortality events (inds m^{-2})	Harvested area (ha)	Previous amount of the stock (10^6 inds)	Local mortality*	Remained amount of the stock (10^6 inds)	Total mortality
Sanbanze	467	526	2,456	.38	1,523	
Kisarazu	439	707	3,104	0	3,104	
Futtsu	330	71	234	0	234	
Total			5,794		4,861	.16

* Mortalities in the areas except Sanbanze were assumed to be 0

b. River flood in Sanbanze in September 2007

Local area	Clam density before the mortality events (inds m^{-2})	Harvested area (ha)	Previous amount of the stock (10^6 inds)	Local mortality	Remained amount of the stock (10^6 inds)	Total mortality
Sanbanze	287	526	1,510	.19	1,223	
Kisarazu	308	707	2,178	0	2,178	
Futtsu	214	71	152	0	152	
Total			3,840		3,553	.07

c. Winter wave mortality in three areas in October 2009–February 2010

Local area	Clam density before the mortality events (inds m^{-2})	Harvested area (ha)	Previous amount of the stock (10^6 inds)	Local mortality	Remained amount of the stock (10^6 inds)	Total mortality
Sanbanze	416	526	2,188	.98	44	
Kisarazu	235	707	1,661	.89	183	
Futtsu	214	71	152	.93	11	
Total			4,001		198	.95

d. Sea spider in Kisarazu in June–August 2009

Local area	Clam density before the mortality events (inds m^{-2})	Harvested area (ha)	Previous amount of the stock (10^6 inds)	Local mortality	Remained amount of the stock (10^6 inds)	Total mortality
Sanbanze	141	526	742	0	742	
Kisarazu	388	707	2,743	.38	1,701	
Futtsu	32	71	23	0	23	
Total			3,508		2,466	.30

(Table 2b–d). The largest impact for the asari stock in Tokyo Bay was estimated to be given by winter wave mortality.

The factors related to the decline of asari stock can be characterized in terms of some aspects; *i.e.*, spatial extension of the effects, intensity of mortal pressure, continuity and length of affected period, repeatability, and time occasion of occurrence. (Table 3) Comparing these characterized properties and estimated impacts of exampled events among the examined factors, the factor which affected wider areas and longer period with higher mortality was supposed to give severer impact for the asari stock. In the estimated factors, winter wave mortality belonged to the factors of severe impact. Although the impact of hypoxia on larval survival could not be estimated due to limited quantitative data, this factor seemed to be included in the factors of severe impact according to above characterization.

In this presentation, the asari stock in Tokyo Bay as an example, we compared the estimated impacts of four factors which have been indicated to be responsible for the asari decline. Present estimation should be required further detailed quantitative data because it was based on the data of the clam size of larger than 2 mm mesh. However, we consider that present estimation could illustrate an outline of the significance of the impacts of the factors. Acquisition of quantitative data and estimation of impact is to perform also with the other factors which did not discuss in this presentation due to insufficient data. In order to take effective countermeasures for the decline of asari in Japan, based on the precise determination of the cause of the decline, further assessment of the impact of the suspected factors is required to progress.

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Table 3. Comparison of the properties of the factors affected on the mortality of the clam stock in Tokyo Bay.

Property	Blue tide	River flood	Winter wave mortality	Sea spider	Hypoxia on larvae
Spatial extension	Partial	Partial	Whole area	Partial	Whole area
Mortal pressure	Partial	Partial	Near Whole	Partial	?
Continuity	Transient	Transient	Successive	Transient?	Successive
Affected period	Short	Short	Long	Long?	Long
Repeatability	Repeatable	Repeatable	Repeatable	?	Repeatable
Time occasion	Irregularly	Irregularly	Regularly	Irregularly	Regularly
Exampled impact	0.16	0.07	0.95	0.30	?
Evaluation	Light	Light	Severe	Light	?

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Perkinsus olseni (Lester and Davis 1981) infection in the Manila clam (*Ruditapes philippinarum*) in Korea; species identification, impacts and spatio-temporal distribution

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Abstract: Asari or Manila clam *Ruditapes philippinarum* (=*Venerupis philippinarum*) is commonly distributed on the west and south coast of Korea, supporting the local shellfisheries industry. Studies examined Manila clams in Korean waters have reported Perkinsosis, a parasitic disease caused by protozoan belonging to the Genus *Perkinsus*. PCR analysis of the Perkinsosis agent indicated that *P. olseni* is only the Perkinsosis agent in Manila clam in Korean waters, while *P. houshuensis*, first discovered in Japan from Japanese Manila clam, detected from *R. variagata* in coastal Jeju Island. Several surveys carried out in Korean waters for the past decade revealed that infection intensity of *P. olseni* over $1x10^6$ cells/g tissue caused necrosis and lesion of the gills and the mantle tissues. Heavy infection with *P. olseni* also retarded reproductive process of the clams, including slow gonad maturation and decrease in the reproductive effort. Clams survived from mass mortality event occurred during post-spawning period on the west coast also showed high infection prevalence and intensity of *P. olseni*, suggested that *P. olseni* is closely linked to the mass mortality event. Despite the high infection intensity, some clam populations on the coastal Yellow Sea where the phytoplankton biomass was relatively higher, showed relatively higher condition factor, suggested that high level of food supply may compensate the energetic loss caused by high load of the parasite in Manila clam.

Key words: *Ruditapes philippinarum*, *Perkinsus*, parasitic disease, food condition factor

Endemic to the western Pacific, Manila clam or Asari *Ruditapes phillipinarum* is commonly distributed on the West and South coast of Korea, supporting the local shellfisheries industry. For the past two decades, the annual landings of Manila clams in Korea have declined due to decrease in the population size, although the main cause of this decrease remains unclear. Several studies have reported that recurring mass mortalities of Manila clam on the west coast seems one of the reasons attributable to the decline of the population size and the mass mortality events were focalized to certain areas, where Manila clams have been cultured intensively for the past several decades. Field surveys carried out during the mortality events suggested that the clam mass mortality is in part, associated with a protozoan parasite, *Perkinsus olseni*. The level of *P. olseni* infection among clams from the west coast often exceeded over million cells per gram tissue, considered to be high enough to evoke Perkinsosis, a parasite disease caused by the protozoan parasite belonging to the Genus *Perkinsus*.

Species of genus *Perkinsus* have been reported to cause mass mortalities in cultured and wild mollusks throughout the world. *P. marinus* has affected the oyster *Crassostrea virginica* populations of the USA, causing mass mortality and dramatic economic losses (Andrews, 1996). *P. qugwadii* was identified from Japanese scallop *Mizuhopecten yessoensis*, which was introduced from Japan to west Canada,

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and associated with mortality of the hosts (Bower *et al.*, 1998). *P. olseni* was blamed for mortalities of abalone species *Haliotis* spp. in Australia and New Zealand and clams *R. philippinarum* and *R. decussatus* in Europe (Goggin and Lester, 1995; Choi and Park, 2010). Moreover, *P. olseni* may be playing a major role in the decrease in Manila clam recourse in Japan because high infection levels in clam populations that have suffered decline in population size. *P. honshuensis* was first discovered in Japan from Manila clam (Dungan and Reece, 2006), although it was not found from Manila clam population in Korea. *Perkinsus* species, except for *P. qugwadi*, have propagation phase in host tissue and free living phase in seawater in their life cycle (Fig. 1). Trophozoites are the propagative stage living in host tissue. Death of the host results in anaerobic condition which stimulates the growth of trophozoites into prezoosporangia. When they are exposed to seawater, prezoosporangia become zoosporangia, which release the infective stage, zoospores.

Current Status of Perkinsosis in Korean Waters

In Korea, *Perkinsus* infection was first discovered and reported from Manila clam population in Gomso bay, off the west coast by Choi and Park (1997). PCR analysis (ITS, 2, NTS and 5.8rRNA sequences) of the Perkinsosis agent found from Manila clams revealed 99.9% genetic similarity between other *P. olseni* strains, indicating that *P. olseni* is the Perkinsosis agent in Manila clams in Korean waters (Park *et al.*, 2005). Park *et al.* (1999) investigated

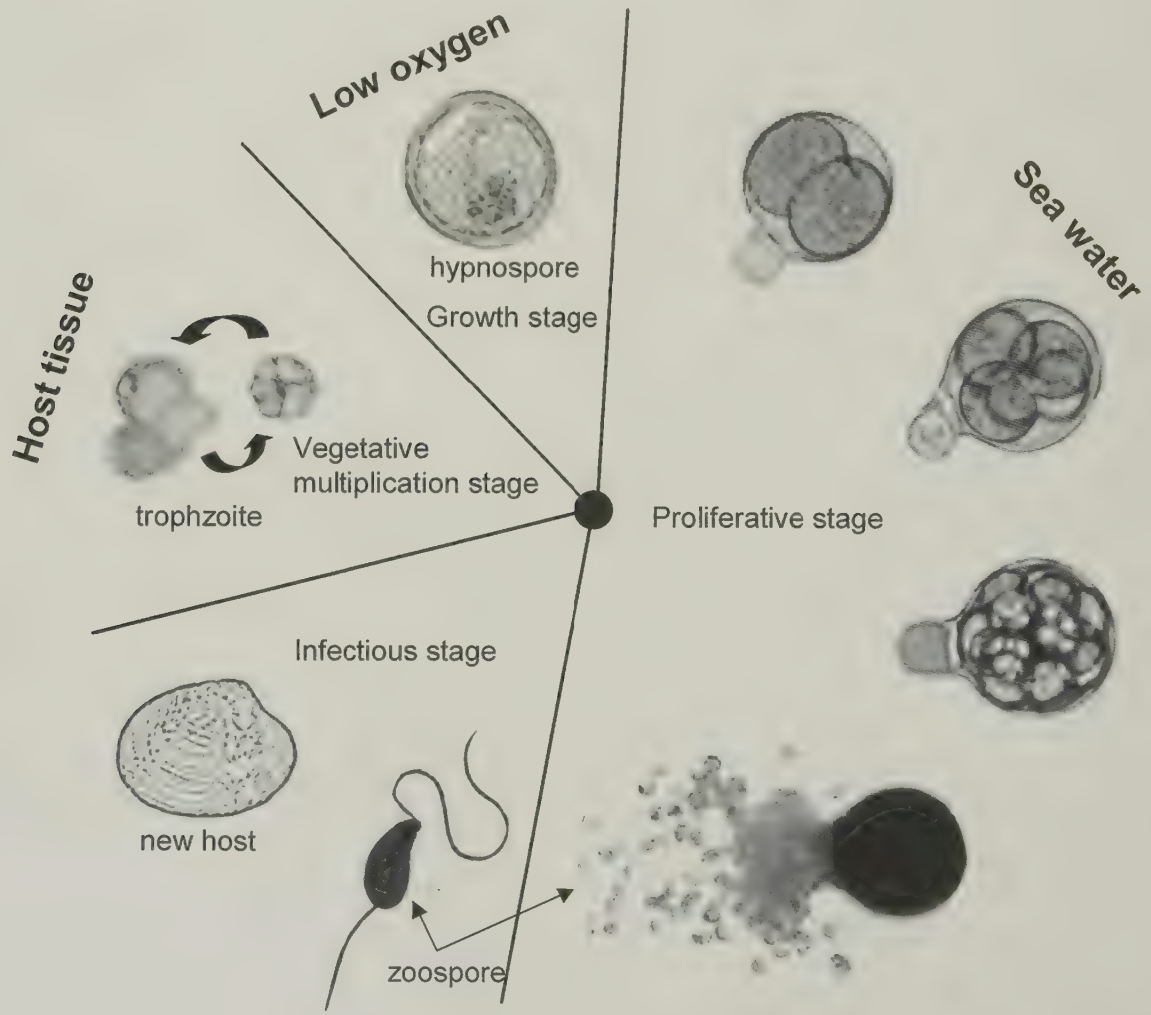

Fig. 1. Lifecycle of *Perkinsus olseni* infect Manila clam *Ruditapes philippinarum* (from Choi and Park, 2010).

P. olseni infection in Manila clam population in Gomso Bay, where recurrent mass mortalities of the clams occurred during post spawning season in fall. The authors reported 100% of prevalence and high infection intensities (up to 2,000,000 cells/g wet tissue) from the clams and suggested heavy infection with *P. olseni* the mass mortalities. Park and Choi (2001) first surveyed *P. olseni* infections in Manila clam populations on the west, south, east and in Jeju Island (Fig. 2). The authors surveyed the infection intensity and prevalence of *P. olseni* from 22 locations using Ray's fluid thioglycollate medium assay (RFTM). As the data revealed, the heavily infected clam populations were concentrated on sandy-mud tidal flats on the west coast, where the tidal flats have been used as a culture ground for the past decades. It was also noticeable that no *P. olseni* infection was observed from clam populations in sand beaches on the east coast and north Jeju Island. The survey suggested that environmental conditions, such as sediment types, salinity and nutrient in the water, determine *P. olseni* infection.

Several studies carried out in Korean waters surveying Perkinsosis for the past decade revealed that heavy infection with *P. olseni* cause necrosis and lesion of the gills and the mantle tissues (Park and Choi, 2010). Such a heavy infection in gill tissues would deteriorate filtration activity, resulting in decline in growth and condition of the host. Heavy infection with *P. olseni* also retarded reproductive process of Manila clams, including slow gonad maturation and decline in the reproductive effort. Park *et al.* (2006) first investigated impacts of *P. olseni* infection on egg production of adult Manila clams in Gosoe Bay, Korea using ELISA, which were developed by Park and Choi (2004). In their study, the amount of egg in an individual clam was negatively correlated with *P. olseni* infection, demonstrating that more heavily infected females produced less amount of egg during spawning season (Fig. 3). Histology also revealed that *P. olseni* trophozoites were commonly found in the connective tissues of female and male gonad (Park and Choi, 2001; Choi *et al.*, 2002), suggested that *P. olseni* interfered the host reproduction. Those data clearly demonstrated that higher level of *P. olseni* infection deteriorates reproduction of clams, resulting in decline in Manila clam populations on the tidal flats in Korea.

The National Fisheries Research and Development

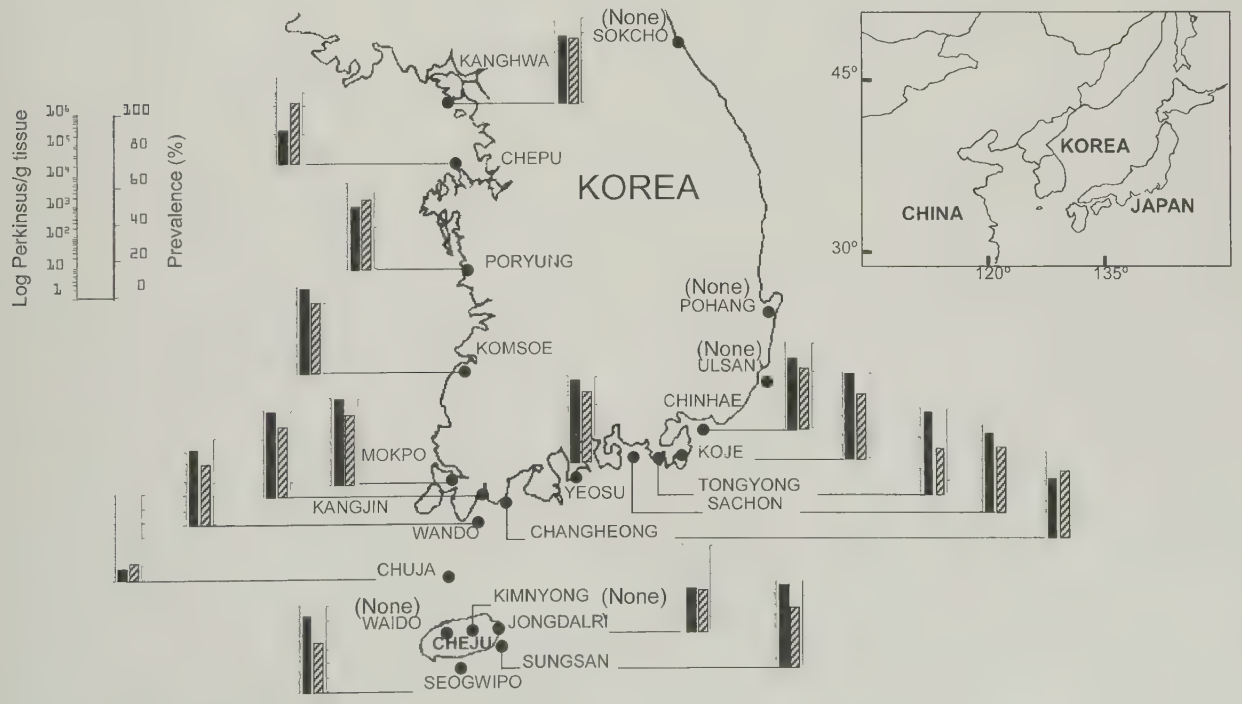

Fig. 2. Mean infection intensity (cells/g wet tissue) and prevalence of *Perkinsus olseni* in Manila clam population in Korea (from Park and Choi, 2001).

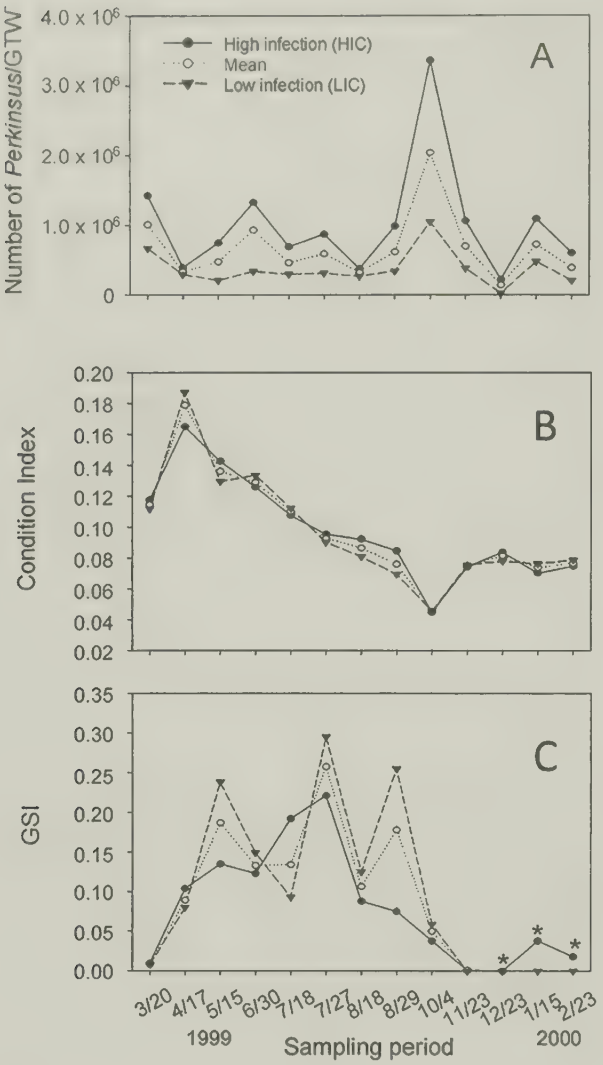

Fig. 3. Seasonal changes in *Perkinsus* infection, protein concentration, condition index and GSI in Gomso Bay, Korea. GTWT represents gram tissue wet weight of Manila clams (from Park *et al.*, 2006).

Institute (NFRDI) of Korea have reported mass mortalities of Manila clams occurred recurrently from clam culture grounds on the west coast of Korea for the past decade. According to NFRDI, the mass mortalities of Manila clam mostly occurred in Incheon Bay and Taean area in early spring and fall. In particular, the mass mortality of Manila clam occurred during fall was likely associated with high level of *P. olseni* infection and post-spawning period in some major clam culture grounds. According to Park and Choi (2001) and Park *et al.* (2006), most of adult clams at major clam culture grounds in Taean and Gomso Bay exhibit high level of *P. olseni* infection and the parasite numbers exceed 10^6 cells/g wet tissue. Waki and Yoshinaga (2013) demonstrated that a cultured strain of *P. olseni* became lethal to Manila clams when geometric mean infection intensities reached ca. 10^6 cells/g wet tissue in juvenile (ca. 5 mm in shell length) and adult Manila clams (ca. 20 mm in shell length). Interestingly, the infection intensity of *Perkinsus* was ca. 10^6 cells/g wet tissue in wild Manila clam populations with the highest infection levels in Korea (Park and Choi, 2001), suggested that *P. olseni* appeared to have had a significant effect on the survival of wild Manila clams in Korea.

Despite the high infection intensity, some clam populations on the coastal Yellow Sea, where the phytoplankton biomass was relatively higher, showed relatively higher condition factor. Accordingly, it is believed that that high level of food supply in these areas may compensate the energetic loss caused by high load of *P. olseni* in Manila clam, resulting in high condition factor. Previous studies have reported that condition of Manila clams is determined by several biotic and abiotic factors, including water temperature, salinity, and level of available food in the environment. As the data showed, Manila clam condition is determined not only by the parasite load including *P. olseni*, but also by environment factors, especially by level food supply.

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Development of Manila Clam Industry in China

Jianguang FANG*1 and Zhihua LIN*2

Abstract: Over 2 million tons of Manila clams *Ruditapes philippinarum* (Adams et Reeve) are produced each year on the coast of yellow Sea, Bohai Sea and East China Sea in China. Most of the production occurs in Liaoning, Shandong and Fujian Provinces. Since 1990's, the Manila clam industry has developed rapidly in China prompted by the demand from domestic and abroad. In order to meet the increasing demand of seed from the whole China, the seed production in reclamation areas became the major sources for Manila clam farming in China. Meanwhile, the Manila clam is also one of the major species farmed with shrimp, fish and crab in form of pond integrated multi-trophic aquaculture which is practiced in commercial scale in Zhejiang and Fujian Provinces. The farming areas of the clam has extended from intertidal zone to shallow sea within 10 m depth along the coast of Shandong and Liaoning provinces. Benefiting from longer time immersing in water and abundant phytoplankton supply, the clam cultivated in deeper water has a much higher growth rate and longer suitable harvest time than that of the clam growing in natural inhabits. One of the main problems for such clam farming is the predation from crab, starfish and sea snails.

Key words: Manila clam, seed production, pond farming

China ranks first in mariculture production in the world, and the annual production reached 18,126,481 tons in 2014, with 13,165,511 tons of shellfish production which accounts for about 72.6% of the total production (Fig. 1). Among the shellfish aquaculture, oyster leads all other species with the total annual production of 4.35 million tons in wet weight with shell. Clams rank second with the total annual production of 3.97 million tons in wet weight with shell (Fig. 2).

The production of Manila clam *Ruditapes philippinarum* (Adams et Reeve) was mainly from the capture fishery before 1980's with the natural seeds in China. After 1980's, aquaculture of this

Fig. 1. Annual mariculture production of China in 2014.

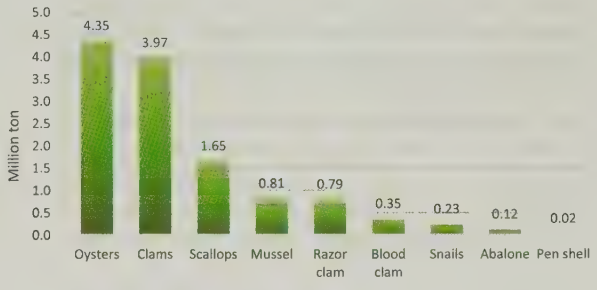

Fig. 2. Annual production of different species of cultured shellfish in 2014.

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species developed rapidly as a result of increasing demand from both domestic and international market. Now, over 2 million tons of Manila clams are produced each year along the coast of Yellow Sea, Bohai Sea and East China Sea in China. Most of the production occurs in Liaoning, Hebei, Shandong, Zhejiang and Fujian Provinces.

Before 1990's, most Manila clam was harvested by manpower from natural muddy flats. Nowadays, in Northern China, the Manila clam farming areas extended from muddy flat to shallow sea within 10 m depth and harvested by dredge boat. In southern China, pond is the major field for Manila clam aquaculture, especially integrated multi-trophic aquaculture with shrimps and fish.

Seed production

Before 1980's, all farmed Manila clams relied on natural seeds. Farmers collected seeds from the natural inhabit and transported to suitable areas for releasing. Since 1980's, the Manila clam industry has developed rapidly in China prompted by the increasing demand domestically and internationally. In order to meet the increasing seed requirement, the seed of Manila clam produced in reclamation areas in Fujian Province became the major sources (Fig. 3).

Seed production in hatchery was started in the mid 1990's in Liaoning Province, northern China, with about 50 billion juveniles in 4 mm shell length annually. But it has declined gradually in both production and scale, mainly due to the higher cost compared to that in reclamation pond in Southern China (Fig. 4). Nowadays, about 80% of Manila clam seeds for aquaculture in China are produced in reclamation areas (big pond with the area of 100 ha) in Fujian Province. The rest is from natural seed habitat

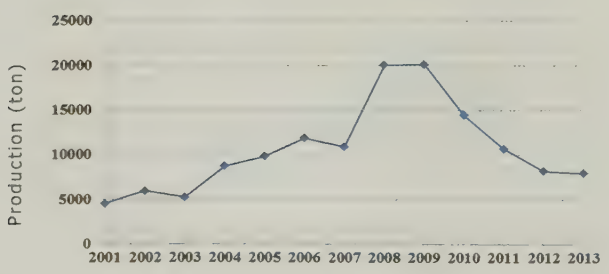

Fig. 3. Seed production of Manila clam in Fujian Province from 2001-2013.
The seeds are cultured in reclamation ponds up to the shell length of 4 mm.

High density culture system for Manila clam seed production was also developed in China around 2010 in Qingdao, Shandong Province. The up-welling system is utilized for larvae culture with the density of 100-150 ind/mL (Fig. 5a). When larvae reach 200 μm, the eye spot larvae are moved from the up-welling system to big tanks for settlement. When spats reach 500 μm, they are collected and moved to spat up-welling or race way nursery sets until commercial seed size in shell length of 5 mm (Fig. 5b).

High density culture system for Manila clam seed production is efficient in laborsaving and space utilization of hatchery; however, the total cost is still much higher than that produced in reclamation ponds. Therefore, the high density culture system has not been practiced in commercial scale for Manila clam seed production in China yet.

Aquaculture

The farming of Manila clam was mainly carried out in tidal zone and subtidal zone before 1980's. A continuous growing domestic and overseas demand pushed the attempts to increase the production of Manila clam in early 1990's. The farming areas of the clam have extended from intertidal zone to shallow sea within the depth of about 10 m along the coast of Shandong and Liaoning Provinces. Benefiting from longer time immersing in water and abundant phytoplankton supplement, the clam cultivated in deeper water has a much higher growth rate and longer suitable harvest time than that of clam in natural inhabits. One of the main problems for such farming is the predation from crab, starfish and sea snails.

Normally, the aquaculture procedures of Manila clam in shallow sea are as follows.

- Removal of the non-alive shells from planned aquaculture area
- Sowing of the seed in spring or autumn at the density of 500-1000 ind/m^2 depended on the

Fig. 4. Clam seed hatchery in Liaoning Province, Northern China.
a: outside view; b: inside view; c: larvae and spat culture tanks; d: indoor tanks at spat nursery facilities.

Fig. 5. Photographs of high density culture system for Manila clam seed in Shandong Province, Northern China.
a: high density facilities for larval culture; b: upwelling and race way nursery sets for spat culture.

seed size and bottom conditions

- Farming for about 15 to 20 months from the seed to marketable size
- Regular removal of the predators such as starfish, snail, and crab during the farming periods by trap nets
- Selective harvest depending on the market demand when the shell length reaches 3 cm
- The mean yields of marketable clams can reach 2-3 kg/m^2 in the mudflat, and 3-5 kg/m^2 in deeper waters.

Because of low temperature, the clam stops growing from December to early March. In order to enhance the profit of the clam aquaculture, a

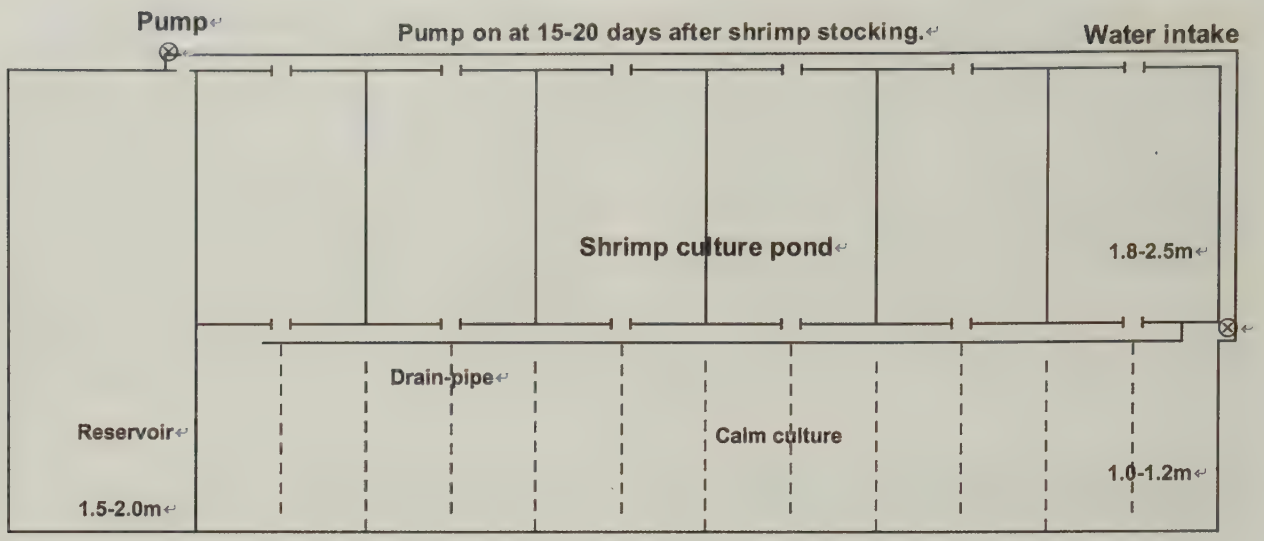

Fig. 6. Schematic diagram of the recirculating system for shrimp and clam culture in Zhejiang Province.

Fig. 7. The photo of integrated aquaculture pond in Zhejiang Province, China.

new method has been practiced by the farmers in northern China as follows.

- Purchase of the seed from Fujian Province in autumn with shell length of 5 mm
- Nursing the seed in indoor or special pond, and over-wintering in green-house for continuous growing of the clam in cold season
- Transfer of the juvenile clam from green-house to the mudflat for intensive grow-out in the following April
- Harvest of the clams with shell length of 3.0 - 3.5 cm within 10 - 14 months from the spawning.

With this method, culturing period can be reduced by about 5 months compared with the traditional method.

In southern China, mainly in Zhejiang and Fujian Provinces, the recirculating culture of the clams with shrimp has been well developed (Fig. 6). In this aquaculture system, the area ratio of shrimp pond to mollusk pond is 10:4. The water recirculation ratio is increased gradually from 10%/d in the early to 40%/d in the late stage.

Recently, the integrated aquaculture system has been well developed in the re-constructed pond in Zhejiang and Fujian Provinces (Fig. 7). In this system, filter-feeding bivalves and predatory animals are separated by a net enclosure in the purpose of preventing predating. In the integrated aquaculture pond, shrimp is stocked at the density of 30 ind/m^2 at the postlarval stage, while clams are normally stocked from March to April at the density of 150 - 200 ind/m^2 with size of 300 ind/kg. Before stocking, the water in the pond is fertilized with well-fermented organic fertilizer for maintaining the water transparency to 30 - 40 cm with yellowish or light brown color during the cultivating period, and exchange the water by 5 - 10 cm/d in the late period. The main species for integrated pond aquaculture and the economic analysis in Zhejiang and Fujian Provinces are listed in Fig. 8 and Table 1.

Problems and challenges for Manila clam aquaculture in China

First of all, environmental pollution from land

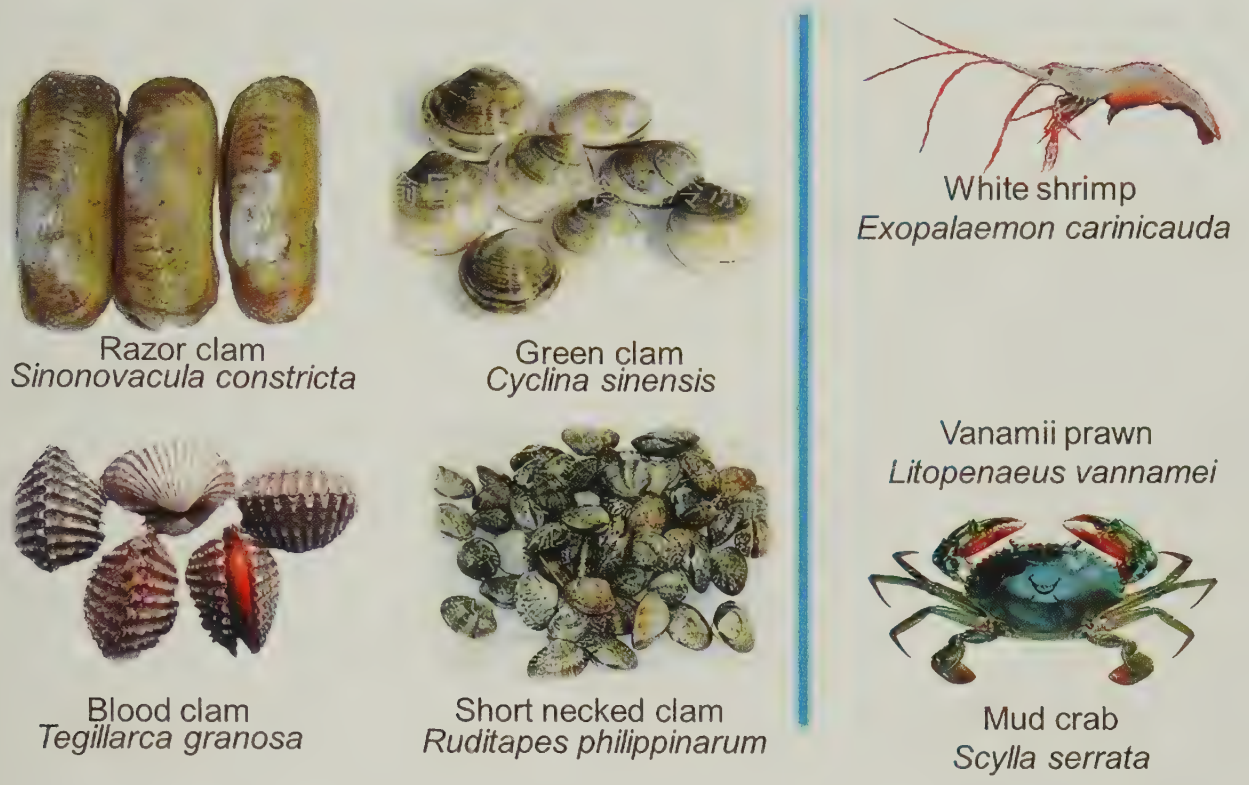

Fig. 8. The main species for integrated pond aquaculture in Zhejiang and Fujian Provinces.

Table 1. Comparison of mono- and integrated aquaculture models in ponds

	Mollusk culture	Shrimp culture	Crab culture	Integrated culture	Recirculating culture
Cost (10^3Yuan/ha)	7.5–10.5	13.5–19.5	16.5–19.5	10.5–15.0	10.5–15.0
Profit (10^3Yuan/ha)	6.0–7.5	10.5–15.0	10.5–13.5	13.5–18.0	12.0–18.0
Risk	Middle	High	Middle	Low	Low
Pollution	Light	Severe	Severe	Light	Light

industry and agriculture is one of the major threats to mariculture, which not only causes the frequent disease outbreaks but also affects the yields and seafood quality.

Secondly, the ecological hazards occur frequently in recent years, such as red tide, green tide and breakout of starfish, which seriously hamper the development of clam aquaculture.

The most serious problem or challenge for the sustainable development of clam aquaculture is the farming space, which has been decreasing mainly due to coastal reclamation. Traditional mariculture areas are encroached by the development of industry.

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Asari clam (*Ruditapes philippinarum*) in France: history of an exotic species 1972 - 2015

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Abstract: In 1972, France underwent an oyster (*Crassostrea angulata*) crisis and urgently needed to diversify its aquaculture. Thus, Asari clam (*Ruditapes philippinarum*) was introduced at that time for aquaculture purpose, concomitantly with the introduction of Pacific oyster (*Crassostrea gigas*). All Asari clam adults and spat originated from the same area (Puget Sound, WA, USA). After a promising start and the implementation of a national Research & Development program, Asari clam culture rapidly faced a series of concomitant handicaps: firstly, in spite the fact that cultural practices were optimized and locking points addressed, leasing ground availability was limited during the 1980s' due to certain reluctance from oyster farmers to share their leases and/or diversify their activity; secondly, mortality events in the parks probably due to the spat quality, diseases and/or zootechnical errors and/or predation by triggerfish; and thirdly economical competition with Italian production rapidly increased, exacerbated by the occurrence of neonaturalized Asari clam populations and the resulting professional fishing. Meanwhile, European vs national regulations, concerning minimum legal shell length of clams devoted to the market, were unfavourable to France due to contrasted rules between the Mediterranean Sea and the Atlantic Ocean; eventually, several diseases impacted drastically clam populations. At the end of the 80's in Northern part of Brittany (France), mass mortality occurred due to brown ring disease, later related to a prokaryote (*Vibrio tapetis*). Presently, the French production remains limited to 2 - 3000 metric tons, mainly based upon professional fishing on neonaturalized populations located in two sites (Arcachon bay and Morbihan Gulf). In Arcachon bay, a comprehensive population dynamics study demonstrated the concomitant effects of fishing activity and environmental characteristics on the population dynamics. A management model was developed to assess various scenarios mainly based on conservation measures (*i.e.* fishing area, and/or fishing licences number, and/or fishing period). Implementation of those recommendations has provided some encouraging results. However, Asari clam fitness remains poor: a genetic impoverishment due to population isolation was argued by fishermen, but transplant experiments demonstrated that these bivalves kept their plasticity, at least in terms of growth and condition index. Thus, several environmental factors were investigated as possible key parameters explaining low clam performances. Again, pathologies were pointed out and particularly the high pressure exerted by the protist *Perkinsus* sp. (perkinsosis). Moreover, a new pathology was discovered in 2005 in Arcachon bay, the brown ring disease. Although the etiological factor has not been confirmed, viral origin is suspected. A meta-analysis comparing Asari clam characteristics in Arcachon Bay with the international literature pointed out that their reduced condition index in this bay was likely resulting from combined unfavorable factors (e.g., diseases, trace elements). However, 30% of the condition index variability among sites at the worldwide level was explained by food availability (chlorophyll *a* concentration). A comparative morphometric studies on four populations of the French Atlantic coasts, using conventional shape analysis, also revealed significant relationships between morphometric ratios

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and environmental parameters (chlorophyll *a* concentrations and seawater temperature). Eventually, marine ecologists and state managers as well, must deal with conflictual goals: on one hand Asari clam is an important exploited bivalve in France, on the other hand, the species remains an exotic species which needs careful attention (e.g., European regulations on invasive species).

Key words: *Ruditapes philippinarum*, France, aquaculture, professional fishing, diseases, environmental stressors

The context of the Asari clam introduction

The decision to introduce Asari (Manila) clam *Ruditapes philippinarum* in France in the 1970's was taken in a particular national context. Firstly, France had a long expertise in shellfish aquaculture since the beginning of the oyster farming in the 1860's. The French shellfish production has been mainly focused on oysters, the native European flat oyster *Ostrea edulis* (1860–1910), the Portuguese oyster *Crassostrea angulata* (1910–1970) and since then the Pacific oyster *C. gigas* (Goulletquer and Héral, 1997). In 1967, oyster farming represented 66% of shell production in France (55,450 t) (FAO, 2014). Thus, State organization (e.g., leasing ground system), infrastructures (e.g., grow out facilities) and technologies (*e.g.* hatcheries) provided favorable conditions to new bivalve species development (Flassch and Leborgne, 1992). Secondly, historical background of French shellfish production, mainly based upon a monoculture approach, therefore showing a weakness when facing disease outbreak, has prompted the State managers to facilitate shellfish diversification. This target was reinforced by severe oyster mortality outbreaks leading to the Portuguese cupped oyster collapse at the beginning of the 1970's (Comps and Bonami, 1977). Moreover, the emergence of Asari clam production in North America since its introduction in 1936 comforted the idea that this bivalve could be a suitable target species considering the wide range of environmental/climatic conditions observed in France.

Asari clam introduction in 1972–1974

Asari clam was introduced between 1972 and 1974 in accordance with 1972 ICES code of practice for the introduction of Non Indigeneous Species (NIS). One consequence was the very little amount of officially imported individuals (all coming from Puget Sound, WA, USA): 500,000 spats, and 1,000 adults (Flassch and Leborgne, 1992) which roughly represented a total biomass of 70 kg. Flassch and Leborgne (1992) suggested that this initial input originated the totality of the European production in 1989 (20,000 t). Today, this strategy provides argument to fishermen that claim that one of the reasons of the actual poor fitness of Asari clam in some parts of France is related to genetic impoverishment. However, two observations mitigate this empiric statement. Firstly, the high level of heterozygoty in French hatcheries (Moraga, 1986; Jeffroy, 2011) and the moderate loss of genetic diversity measured in many European ecosystems (Chiesa *et al.*, 2011; 2014) suggest that unofficial introductions were also carried out in Atlantic and Mediterranean waters. Secondly, transplant experiment between areas showing different environmental characteristics has demonstrated the high growth plasticity of Asari clam: individuals living in adverse situation with poor fitness were able to recover high growth and condition index when transplanted in suitable sites (Dang *et al.*, 2013).

Addressing the technical feasibility and providing advice to shellfish farmers: 1975–1986

The three first years after introduction were mainly devoted to (1) growth performance comparisons with the native species *R. decussatus*, and (2) development and implementation of suitable techniques so as to optimize clam yield. It was clearly demonstrated that the exotic species showed improved performance compared to the European species, therefore prompting the managers to develop a comprehensive Research & Development program focusing on the Asari clam. The first

attempts were successfully achieved in Tudy Island, leading to the first production figures: 125 kg and 1500 kg, in 1977 and 1978 respectively (I.F.R.E.MER,, 1988). Several national R & D scientific programs followed those preliminary field trials in Asari clam production, lasting from 1981 to 1986. Even though positive results were recorded concerning growth performances and spat production in hatcheries, they were mitigated by high spatial variability and high mortality rates in some growing sites, especially when performed in tidal flats (compared to ponds) where temperature variation is high and where hypoxia may occur at low tide (Goulletquer, 1989). High mortality rates were also reported in early spring due to reduced food availability and high turbidity rates over the winter time leading to reduced physiological condition. Asari clam production were first reported in 1982 in the aquaculture national statistics to rapidly reach ca. 500 metric t/y after 1985.

The first serious problems arise (1986-1992)

From 1986, Asari clam aquaculture production reached a plateau at a relatively low level, *i.e.* 500 mt/y. Actually, we can almost state that the story of Asari clam in France was compromised during this period of time due to four main reasons.

Firstly, suitable leasing grounds were already occupied by oyster farmers that were not ready to cease them: in the mid 1980's, out of the 212 km^2 of flats devoted to culture, only 0.03 km^2 concerned Asari clam (I.F.R.E.MER, 1988). Meanwhile, this period of time was marked by oyster overstocking, maximum cupped oyster production and a drastically changing market leading to a precocious shellfish industry. Contrasted performances of Asari clams in France did not motivate oyster farmers to diversify their activity with this new product and placed them in a "wait-and-see" strategy (Robert and Deltreil, 1990).

Secondly, Asari clam populations were severely and rapidly impacted by diseases outbreaks. It started in 1986 with the VTP (vibrio of *Tapes philippinarum*) in hatcheries but this disease was rapidly stamped out. The most emblematic pathology emerged in 1987: the brown ring disease (BRD), being likely partly responsible for the clam aquaculture collapse in Northern Brittany, France (Paillard and Maes, 1990, 1994; Paillard, 2004). BRD was characterized by the occurrence of obvious abnormal conchiolin deposit adhering to the inner shell, obstructing valve closure, and leading to overall clam weakening. The aetiological agent was described 3 years later (*Vibrio tapetis*). Rapidly, BRD was also described in Italy (1993), Spain (1993), Ireland (1998), United Kingdom (1998), Norway (2008) likely due to shellfish transfers among European countries. Then, BRD progressively reached other parts of the world in Asia (Park *et al.*, 2006; Paillard *et al.*, 2008; Matsuyama *et al.*, 2010). A herpes-like virus has also been detected in the context of larval mortality in a French hatchery in 1997 (Renault *et al.* 2001). Subsequent molecular works showed that this virus was the same as OsHV-1 infecting *Crassostrea gigas* (Arzul *et al.*, 2001).

Thirdly, shell farmers had to cope with mortality events (in spring and autumn 1992) that affected 2/3 of the parks. Those mortalities were probably due to the spat quality and/or zootechnical errors and/or predation by triggerfish (Auby, 1993).

Finally, French clam aquaculture rapidly underwent severe economic competition with the naturally production, in France (Gulf of Morbihan and Arcachon) but also especially with the Italian products. Rapidly, neonaturalized populations occurred in Italy (Venice lagoon, Sacca di Goro) leading to large professional fishing and an economic competition between aquaculture and commercial fishing. Moreover, European regulation implemented a different capture length for Atlantic and Mediterranean sectors, 35 mm (40 mm from 1998 to 2008) and 25 mm shell length, respectively. This prompted to economic discrepancies with Italy and more generally Mediterranean products because France had to homogenize this parameter for its whole territory and had to select the most restrictive value (35-mm minimum shell length). Eventually, neonaturalized Asari clam populations also occurred in France (e.g. in 1988 in Arcachon Bay) and national commercial fishing production (1,500 t) was already three times the national aquaculture production in 1990. In this context, the clam exchange value dropped from the equivalent of

8.4 €/kg (1120 ¥/kg) to the equivalent of 6.1 €/kg (820 ¥/kg) in 1988 and 1989 respectively – presently the exchange value is ca. 2.5 €/kg (330 ¥/kg).

As a result, from the 12 hatcheries producing clam in 1987, there is still only the SATMAR (Société ATlantique de MARiculture) in France in 2015.

Relative stagnation of the production (1992–2015)

Unfortunately reliable long-term statistics of Asari production are unavailable at the national level (Caill-Milly, 2012), due at least to three main reasons: 1) the precise identification (*R. philippinarum vs. R. decussatus*) is not compulsory for landings; 2) the difficulty in discriminating between aquaculture and harvesting production from different professional status (fishermen, shellfish farmers who may present a fishing activity); as well as 3) the reform of the French system in charge of commercial catch statistic that occurred in 2008. However, over the last years, we can consider that 50% of the production is provided by aquaculture and the remaining 50% by commercial fishing to reach an overall yearly production of ca. 2,500–3,500 t (Agreste, 2015). Most of the aquaculture production is economically sustainable by private companies having a full control over the clam life cycle – from hatchery production to marketable size. In France, this production is rather disseminated into a limited number of production units, e.g. Chausey Islands (Northern Brittany) is a leading French rearing area with 120–210 t/y (Toupoint *et al.*, 2008; Ifremer 2015). With regard to commercial fishing, 80% of the production is carried out from two areas, Arcachon bay (ca. 500 t) (Southwest Atlantic coastline) and Morbihan Gulf (ca. 300 t) (Southern Brittany)– Those both production areas benefite from regular stock assessments (started in 2000 for Arcachon (Sanchez *et al.*, 2014), in 2001 for Morbihan Gulf (D'Hardivillé *et al.*, 2014)).

Besides the necessity to monitor an exploited site, stock assessment is a relevant tool to reinforce a management strategy for a sustainable development. Based on stock biomass and shell length distribution, but also on population dynamics parameters like growth performance and natural mortality rates (Dang *et al.*, 2010b), a management model for Asari clam was proposed and developed in the case of Arcachon bay (Bald *et al.*, 2009). The model allows observing the trajectory of Asari clam biomass along years according to various conservation measures parameters (i.e., number of fishing licences, no-take zone superficies, fishing days) and environmental conditions (i.e., sea temperature, trophic resources). An host-pathogen-environment numerical model has been also developed for BRD; simulations using 1℃ and 2℃ increases in temperature showed that climate warming might favor the spread of BRD in France but also in south Atlantic and Mediterranean countries (Paillard *et al.*, 2014).

In France, part of the production stagnation is thought to be related to the limited Asari clams' fitness in several sites, possible making this species more sensitive to infectious diseases. Besides the BRD previously cited, several studies demonstrated that Asari clams have been severely impacted by perkinsosis, a pathology related to high infection by the protozoan *Perkinsus olseni* (Lassalle *et al.*, 2007; Dang *et al.*, 2010a). Recently, *P. chesapeaki* was also recorded in French clam populations (Arzul *et al.*, 2012). *Perkinsus olseni* effects on Asari clams remain poorly documented (Soudant *et al.*, 2013). At sublethal level ($<10^5$ cells/g dry weight of gill), parasite abundance can explain up to 26% of the variability in Asari clam growth in the case of Arcachon Bay population (Dang *et al.*, 2013). Moreover, Asari clams collected at the sediment surface (abnormal position) in this same bay exhibited a 3-fold higher *P. olseni* abundance than individuals buried in the sediment (i.e. normal position) and 27% lower condition index (Binias *et al.*, 2014). Finally, Arcachon bay is also impacted by another endemic disease, the Brown Muscle Disease, first reported in 2005 (Dang *et al.*, 2008; Dang and de Montaudouin, 2009; Dang *et al.*, 2009a; Dang *et al.*, 2009b). This disease affects the posterior adductor muscle to eventually drive the clam to the sediment surface where it rapidly dies. On-going research suggests a viral aetiology (Dang *et al.*, 2009b; Binias, 2013).

A meta-analysis to understand poor Asari clam fitness

Arcachon Bay is the leading area for Asari clam

production based upon commercial fishing although it provides clams of poor condition. In 2014, a meta-analysis based on 41 publications originating from 10 countries, compared Asari clam parameters and environmental characteristics worldwide (de Montaudouin *et al.*, 2016). This large-scale study confirmed that Asari clam condition index (CI=flesh dry weight/shell weight) in Arcachon bay was significantly lower (/2) than in all other rearing areas. Chl *a* concentration in the seawater contributed to 30% of the CI variability, Arcachon bay appearing as an oligo/mesotrophic bay. Considering that morphological characters can be used as proxy of nutritional condition (Watanabe and Katayama, 2010), such role of environmental conditions was also highlighted by Caill-Milly *et al.* (2014). A comparative morphometric studies on four populations of the French Atlantic coasts revealed significant relationships between morphometric ratios (integrating weight density indicators) and environmental parameters (chlorophyll *a* concentrations, seawater temperature). Besides, Arcachon bay displayed higher values for *Perkinsus* abundance and prevalence, and chromium and arsenic flesh concentrations in the flesh. Finally, this is the only ecosystem where Asari clams undergo brown muscle disease.

Conclusion: Asari clam is also an exotic species

Although a distribution overlay with the native clam *R. decussatus* may occur, no study in France claims that Asari clam could be a plague as a direct competitor for other species (Laruelle, 1999) (although there is a suspicion that the parasite *Perkinsus olseni* could have been brought with it). In contrast, Asari clam populations have been a resource for seabird wintering in the English Channel – sustaining bird survival rate (Caldow *et al.*, 2007). Few studies have pointed out that the clam aquaculture may indirectly alter environment through intensive harvesting practices (Toupoint *et al.*, 2008). Since the Asari clam was introduced during the 1970s, there is no specific requirements in response to the 2007 European regulation on using exotics in aquaculture. Therefore, Asari clam is listed in the Annex IV of the Council Regulation (Non-native Species in Aquaculture Risk Assessment Scheme). Meanwhile, risk assessment strategy is considered as a suitable tool to deal with exotic/invasive species management, and prevent side effects from exotics' introduction. A preliminary study has used the Asari clam as a case study at the European level to test the risk assessment Excel-based application AS-ISK[i] (Invertebrate Invasiveness Screening Kit) (Copp *et al.*, 2014a) and assess the Annex IV suitability. With the Asari clam, the overall risk is medium with a mark of 2.1 out of 4. When focusing on four assessment topics which are 1) risk of introduction in unintended locations, 2) risk of establishment, 3) risk of dispersal, and 4) risk of environmental and socio-economic impact (Copp *et al.*, 2014b), marks are 3.0, 1.9, 2.0 and 1.4, respectively. This represents a first estimate of risk – not neutral in spite of the Annex IV – associated to the species transfer to additional countries.

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Manila clam introduction in the Sacca di Goro Lagoon (Northern Italy): ecological implications

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Abstract: The manila clam was introduced in 1986 in the Sacca di Goro lagoon (Po River Delta, northern Italy) and in a few years became one of the backbone of the local economy. Few years later, the lagoon was affected by macroalgal blooms, followed by the biomass breakdown which impacted the clam farming, with mass mortality in summer. From late 1987 to 1994, an intensive, monthly-based monitoring plan of water column was thus performed, aiming at analyzing salinity, dissolved oxygen, chlorophyll a and nutrient patterns in a grid of sampling stations, with respect to hydrodynamics and freshwater inputs. Later on, monitoring of spatial distribution, biomass evolution and ecophysiology of the dominant macroalgal community was performed (1990–1998), together with experiments focusing on macroalgal production and factors controlling macroalgal growth and decay. Furthermore, the role of clams in coupling benthic and pelagic processes was addressed as a possible feedback loop in the lagoonal ecosystem. Regular monitoring was afterwards flanked by an experimental approach aiming at more detailed studies of how clams affect benthic processes, in particular aerobic and anaerobic respiration, nutrient recycling, the regulation of denitrification and the analysis of nitrogen cycling within farmed areas (1993–2005). More recently, different European projects addressed the relationships between environmental and socio-economic issues in the clam farming management. In this review we summarize the main outcomes of these research activities with the overall aim of quantifying, at the lagoon scale, the role of the farmed species as regulators of some of the multiple processes involved in the ecosystem metabolism and biogeochemistry. Special emphasis is given to how clams affect uptake, dissipation and recycling pathways of nutrients, especially nitrogen. Overall, the clam farming system is analyzed for its implications in the whole lagoon metabolism and for possible ecological feedbacks which in turn can impact the local socio-economic system.

Key words: Sacca di Goro, Asari clam, sediments, fluxes, anoxia, macroalgae

Estuarine eutrophication: effects, external and internal causes

Eutrophication of coastal areas has generated a huge research effort worldwide since the late 70's. Nutrient delivery to brackish and shallow marine areas, in particular nitrogen loadings, have stimulated primary production and the input of organic matter to the benthic system, with cascading effects for the biota and the matter cycling (Nixon, 1995). Among recognized and well documented negative effects of eutrophication are the shift of primary producer communities (from macrophytes or microphytobenthos to phytoplankton or macroalgal dominated), increased sediment resuspension, erosion and water turbidity, higher demand of electron acceptors by the benthic compartment, sediment and water anoxia and dystrophy (Cloern, 2001). The mechanisms leading to the latter, extreme event were studied and clarified in detail

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(Viaroli *et al.*, 2008; 2010; Zaldivar *et al.*, 2009). Under eutrophic conditions, labile organic input to sediments exceeds the mineralization capacity of the benthic compartment and decouples reduction and oxidation processes, leading to the accumulation of the end products of anaerobic microbial metabolism. The progressive decrease of dissolved oxygen in the bottom water and sediments, the exhaustion of oxidized Fe and Mn pool and the migration of free sulfides to the interface then occurs (Giordani *et al.*, 1996; Zilius *et al.*, 2015). Sulfides are extremely toxic and may kill phanerogams and macrofauna, hampering their metabolic role, including sediment reworking and oxidation, enhancement of P retention and of N loss via coupled nitrification and denitrification, nutrient uptake at the interface and oxygen production in the proximity of sediments (Nizzoli *et al.*, 2007; Bartoli *et al.*, 2008, 2012). Sulfides may also inhibit the activity of denitrifying bacteria and produce a short circuit within the geochemical P buffer mechanism, resulting in large releases of inorganic N and P from sediments, with a positive feedback for eutrophication phenomena (Bartoli *et al.*, 1996; Giordani *et al.*, 1996; Viaroli *et al.*, 2010; Zilius *et al.*, 2015).

However, eutrophication may also have positive aspects for coastal areas. Early eutrophication phases (the so-called *enrichment stage*) for example result in increased fish biomass, as the energy flow within ecosystems increases and supports higher trophic levels. The growth of mollusks may also be favored by moderate eutrophication due to increased phytoplankton availability (Naylor *et al.* 2000; Newell, 2004).

Nutrient enrichment and mollusks farming in the northern Adriatic lagoons (Italy)

The lagoons of the Po River Delta and of the Northern Adriatic coast of Italy receive heavy loads of nutrients generated in the Po Plain, an area which is heavily exploited for agricultural and industrial activities (Viaroli *et al.*, 2013; 2015). A number of watersheds within the Po River basin are characterized by unbalanced nitrogen (N) budgets, with input terms from manure, synthetic fertilizers and, to a lesser extent, urban wastewaters largely exceeding outputs (N uptake by crops), *sensu* Oenema *et al.* (2003). As a consequence, the risk of diffuse water pollution by the N excess is extremely elevated (Bartoli *et al.*, 2012; Castaldelli *et al.*, 2013). Nitrogen loads generated in the agricultural sectors of the Po basin and transported towards the Adriatic Sea generally exceed the metabolic capacity of the transitional zones, resulting in eutrophic to hypertrophic conditions of brackish areas (Abbiati *et al.*, 2010).

The retrospective analysis of the last three decades of the Sacca di Goro Lagoon allows to review the eutrophication phenomena in a deltaic area receiving nutrient-rich freshwater from a basin under multiple human pressures. In this small lagoon, nutrient enrichment and its effects on organic matter production and final fate (eutrophication) must be considered in a comprehensive manner, together with the analysis of other co-occurring socio-economical and environmental changes. Macroalgal blooms and collapses overlapped drastic changes in the local economy, consequences of the successful introduction in the lagoon of the clam *Ruditapes philippinarum*. This farming activity proved to be one of the most commercially successful introduction of an exotic species in a lagoon environment. Clams farming was linked to the lagoon eutrophication with both positive (fast mollusks growth, high yield) and negative (anoxia and massive death of mollusks) feedbacks. Furthermore, human interventions to protect the commercial activity from dystrophic events (i.e. intervention on sand bars and canals to improve water circulation within the lagoon) had other important consequences on water quality.

The effect of clams farming on the lagoonal environment can be synergic and similar to that of eutrophication as sediment dredging to recover clams destroys meadows of rooted phanerogams, enhances the regeneration of nutrients and sediment resuspension, augments locally the chemical consumption of oxygen and, via the deposition of feces and pseudo feces, results in organic matter input to surface sediments that further enhances sediment oxygen demand and nutrients recycling (Bartoli *et al.*, 2001; Castaldelli *et al.* 2003; Viaroli *et al.* 2003). Molluscs farming can also favor changes in the primary producer community as mollusks

promote water transparency with their filtration activities, removing phytoplankton, and excrete inorganic nutrients readily available to opportunistic and not ingestible macroalgae. Simultaneously, mollusks farming can be antagonist to eutrophication as filter feeders remove large fractions of the particulate matter in the water column and convert nutrients in biomass which is exported from the sites of farming (Viaroli *et al.*, 2010).

In this review we analyse the recent evolution of the Sacca di Goro, linking external nutrient-associated eutrophication with internal changes in the lagoon, after the establishment of mollusks farming, the most relevant economic activity. Special emphasis is given to nitrogen cycling, and in particular to the analysis of assimilative and dissimilative pathways and their regulation by environmental factors.

The Sacca di Goro Lagoon

The Sacca di Goro (44.78–44.83°N and 12.25–12.33 ° E) is a small (26 km^2) and shallow (mean depth ~1.5 m) eutrophic lagoon located in the Southern part of the Po River Delta, in the Province of Ferrara (Fig. 1). The lagoon watershed extends over 860 km^2 and is partially subsident and man-regulated. The lagoon receives freshwater inputs from the Po di Volano and a series of scooping plants which drain the lagoon surroundings. The salinity is dynamically variable as a function of the balance between freshwater and tide-driven marine inflows. The western and eastern corners are influenced by freshwater inputs while the central area is more marine. Additional freshwater inputs are from the Po river through the Po di Goro branch and the river plume which enters into the lagoon from the Adriatic Sea. Sediments are a mosaic of sandy, silty and clayey zones; depending upon currents. Sandy areas are located close to sea mouths, whilst muddy areas are common in the more stagnant eastern corner and central area. In the last decade, dredging activities have increased dramatically, aiming at the replacement of muddy with sandy areas, to expand the lagoon surface suitable for clams farming.

The lagoon is eutrophic and from mid 1980s blooms of green macroalgae from the *Ulva* complex have determined anoxic crises and dystrophic outbreaks in summer. Early ecological studies in this lagoon were started by the Universities of Ferrara and Parma in the late 1980's and were stimulated by the Ferrara Province administration as macroalgal blooms were impacting clams farming, a fast developing economical activity. More details and references on the lagoon hydrology, ecology and pollution are reported by Viaroli *et al.* (2006; 2010).

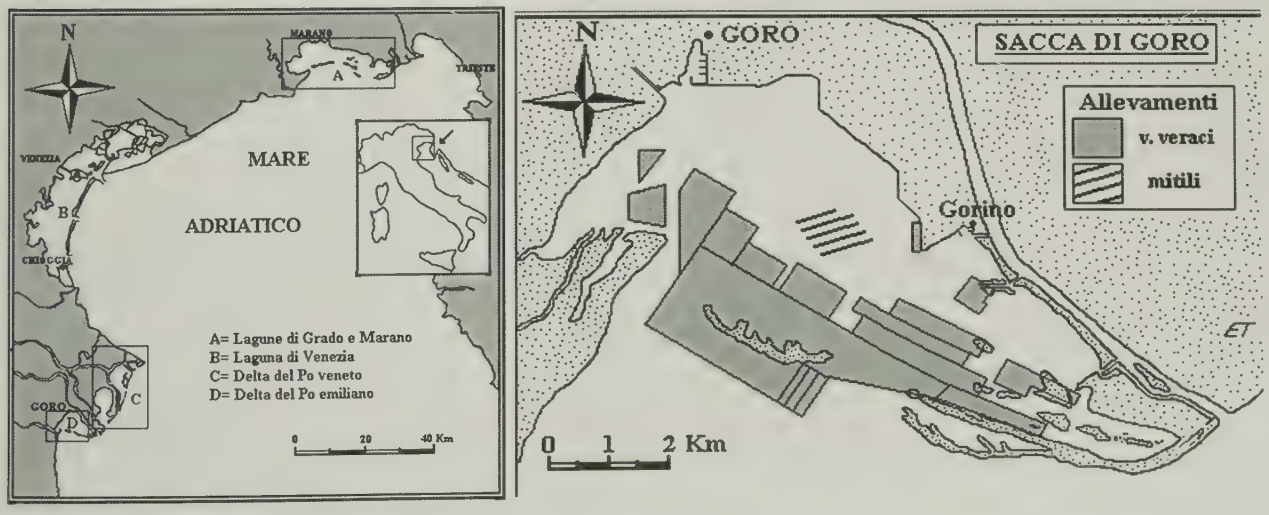

Fig. 1. The north Adriatic coastal lagoons (A=Grado and Marano, B=Venice; C=Po Delta, Veneto Region; D=Sacca di Goro, Emilia Romagna Region) where Tapes philippinarum is farmed. The detailed map of the Sacca di Goro shows areas licensed for clams seeding, in grey.

Ruditapes philippinarum in the Sacca di Goro

The Asari clam was introduced in Italy nearly three decades ago and its farming became rapidly one of the most important economic activity within national aquaculture products. Main sites of production were the lagoons of the Northern Adriatic Sea (Marano-Grado, Venice and those within the Po River Delta, Fig. 1). The production peaked exponentially, reaching in 1999 some 65,000 t yr^{-1}; at present the national production is nearly 35,000 t yr^{-1} (2013) which represents approximately the 70% of the European clam production (Fig. 2). The main reason for such decrease is the much lower contribution of the Venice lagoon to the total, as a consequence of multiple factors (oligotrophication of the lagoon, anoxia, parasites, bad management of the resource). Elsewhere (Goro Lagoon, the study case presented) the production remained stable or even tended to increase due to appropriate management practices, targeting the substrate, water circulation through the tidal canals, the control of macroalgae and the realization of a nursery area in the open sea.

In the Sacca di Goro lagoon clam farming was started in 1986, becoming the main economic activity only five years later. Actually, more than half of the lagoon surface (15 km^2) is exploited (Fig. 1), with an annual production between 15,000 and 18,000 t yr^{-1} (Fig. 2). Local fishermen cooperatives manage the sowing and the harvesting of the clams in well-defined licensed areas. At the beginning of the farming activity, licensed areas were mainly located in the southern area of the lagoon (Fig. 1) where the water exchange rate is higher and the sediments are sandy. Now clams are also sown in muddy substrates, in areas with strong freshwater influence. Young clams (0.5–1.0 cm in shell length) are continuously collected within or in the sand banks just outside the lagoon and immediately sown until they reach the commercial size (2.5–4 cm); in the Sacca di Goro, due to high food availability, this occurs in 8–10 months. More recently, nursery areas outside the lagoon were established in order to provide young clams to fishermen in case of massive death within the lagoon. It is also a common practice to move large amounts of clams from the lagoon to the sea (and vice versa) to avoid loss of product in very dry and hot summers when the risk of

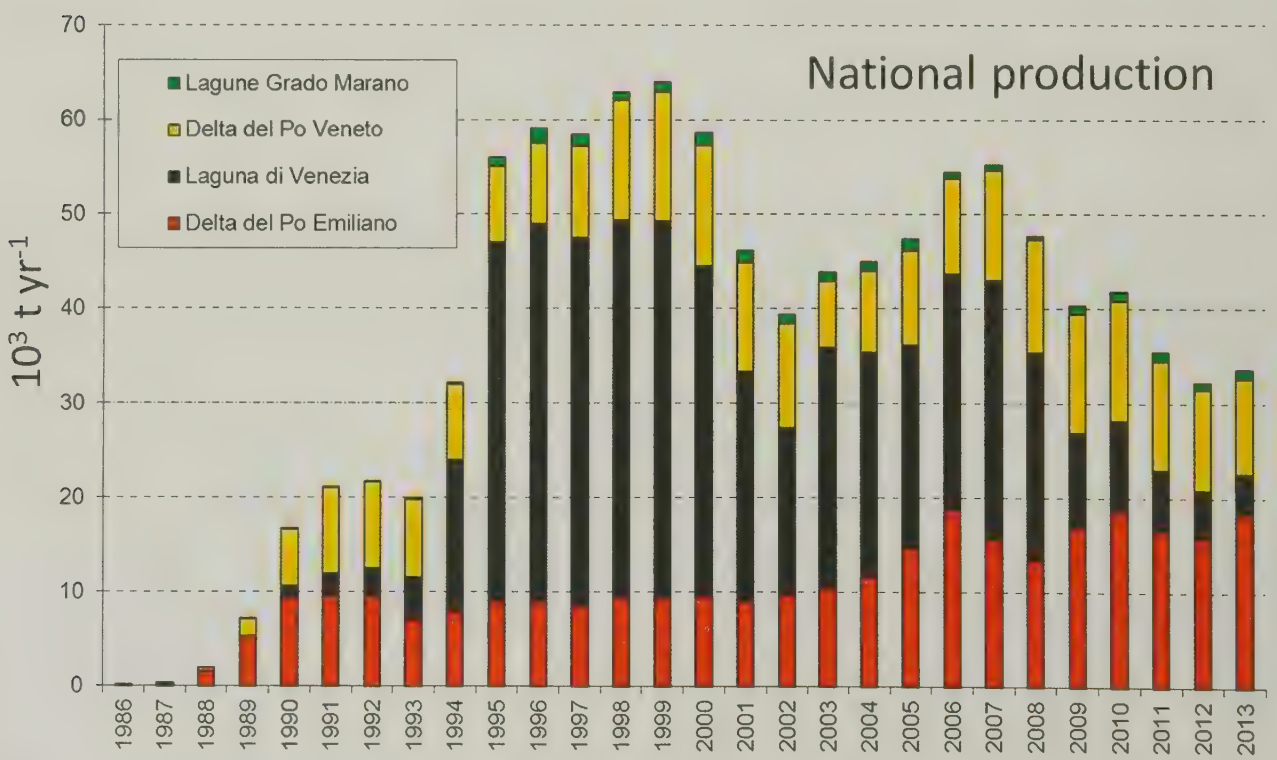

Fig. 2. Temporal evolution of the Asari clam production in Italy. The red bar represents the production within the lagoons of the Emilia Romagna Region, almost entirely represented by the Sacca di Goro.

anoxia is elevated. *Ruditapes* densities are generally maintained at around 1000 adult individuals m^{-2}; but densities may be higher in well flushed areas, with some risks due to their elevated metabolic activity.

Despite its small dimensions, the Sacca di Goro lagoon supports the local economy and provides the main revenue of the resident population, which ranges between 50 and 100 million euros yr^{-1}. Shellfish farming is socially relevant, it ensuring about 1,500 direct job positions, plus employments in seafood industries, commercial and side activities, e.g. shipbuilding (Viaroli *et al.*, 2012). Clam farming is managed by cooperatives of fishermen that access licensed areas, under the control of regional and local authorities. The extension of licensed areas and the product quantity which is daily delivered to the market are controlled by the cooperative consortia in order to guarantee both food quality and adequate revenue. For this reason, each fisherman can harvest a fixed quota that is established day by day, based on the market demand. Harvesting is performed with hydraulic dredging, which causes sediment alteration and resuspension with local effects on water quality (Viaroli *et al.*, 2003; 2010).

The deterioration of water quality has been also claimed as one of the major causes of macroalgal blooms which are often causing serious setbacks due to massive clam mortality, especially in summer (Viaroli *et al.*, 2006). To avoid anoxia the most successful intervention was and still is based on frequent canal dredging to improve water circulation within the lagoon and avoid stagnation.

Research activities in the Sacca di Goro

Water chemistry, development and control of macroalgae: The most complete and continuous water monitoring of the Sacca di Goro Lagoon was performed from 1988 to 1994 with a monthly frequency, at 8 stations, coupled in 4 homogenous sub-areas: the western corner, directly influenced by the Po di Volano, the deeper central part, the southern part, near the sand bank and the sea and the most confined eastern corner, called Valle di Gorino. Despite natural and anthropogenic modifications, and in particular the evolution of the sand bar separating the lagoon from the sea and the network of canals that was created to improve water circulation, this zonation was substantially maintained, with respect to salinity, nutrients, macroalgae, zoobenthos and the risk of hypoxia. The latter always characterized the Valle di Gorino and the central area of the lagoon, which are the areas generally invaded by macroalgae. The zone with highest nutrient and chlorophyll concentrations is that receiving the Po di Volano discharge, showing pronounced seasonal variation with winter peaks and summer minimum. Freshwaters from the Po di Volano and Po di Goro rivers are contaminated by nitrogen, especially by nitrates, which in late winter and spring attain up to 5 mg N L^{-1}, whilst phosphorus concentrations peak in summer with up to 500 μg P L^{-1}, and display average values of ~ 100 μ g P L^{-1}. The nitrogen loading to the lagoon, estimated in 2-3,000 t yr^{-1}, has been considered responsible for the abnormal growth of green nitrophilous seaweeds of the *Ulva* complex and red macroalgae of the *Gracilaria* genus (Viaroli *et al.* 2006). Usually, macroalgal growth starts in the eastern shallow and most confined areas in late winter and the maximum spreading is attained in late spring. Extended *Ulva* blooms have been recorded from 1987 to 1992, in 1997-98 and more recently from 2005 to date. During the bloom peak, half of the lagoon is covered by macroalgae with biomass densities attaining 400-800 g m^{-2} as dry matter. Drifting biomasses often accumulate in the shallower areas, especially in the southern zone where clams concessions form a sort of barrier. In June-July, the sudden collapse of macroalgal mats is followed by biomass decomposition which causes anoxia, bacterial sulphate reduction and sulphide release into the water column. During neap tides and under calm wind conditions, anoxia may persist for several days, causing clams losses up to 30% of the annual production.

In order to either avoid or mitigate the impact of blooms, macroalgal biomass are harvested when attaining thick mats which cause serious threats to clams. However, the effectiveness of macroalgal harvesting seems to be low, because the harvested biomass is only a small fraction of the whole biomass bulk. This is an interesting point in terms of better linking science and management. Studies

were performed in the lagoon demonstrating that to be effective the harvesting rate has to be much greater than macroalgal growth rate, which can be achieved with an optimization of the cost to benefit ratio (Cellina *et al.*, 2003). Nonetheless, macroalgal harvesting is generally designed without taking into account the scientific outcomes. Together with water monitoring, research activities focused on the ecophysiology of *Ulva*. One of the main question was dealing with the capacity of the macroalgae to grow during the late spring-early summer, a period of limited N input from rivers and strong N-limitation. Another question was related to the factors determining the sudden collapse of algal blooms over large lagoon surfaces. Laboratory studies suggested that *Ulva* may incorporate nitrogen (as nitrate) during periods of high availability and store it within the thalli, a capacity that provides a competitive advantage to the macroalga (Naldi and Viaroli, 2002; Viaroli *et al.*, 2005). Regarding the causes of the blooms collapse a univocal answer was not found. It is likely that multiple, co-occurring factors stress the macroalgae, including water stagnation, prolonged nutrient limitation and anoxia and sulfide toxicity. *Ulva* blooms (and collapses) were always related to external factors (as nutrient inputs from rivers) while internal sources as sediments, and in particular sediments with clams, were scarcely considered.

Biogeochemical implications of clams farming: Bartoli *et al.* (2001) evidenced by a simple incubation of intact cores collected in a control and in a farmed area, that sediments with clams can regenerate impressive amounts of inorganic nutrients to the water column and decouple oxygen consumption and carbon dioxide release. These results were basically due to the density of cultivated organisms, generally 10^3 higher that under natural conditions, and to their metabolic activity. With an upscaling procedure, it was demonstrated that mollusks farming in the Sacca di Goro deeply altered the whole lagoon benthic (and pelagic) metabolism, stimulating by a factor 1.8 and 3.3 the sedimentary oxygen consumption and carbon dioxide release, respectively. The proportionally higher stimulation of CO_2 versus O_2 fluxes suggests that the biodeposition of labile organic matter as feces and pseudofeces increases anaerobic metabolism in the Sacca di Goro and the accumulation of chemically reduced compounds within sediments. Clams excretion stimulated also at the whole lagoon scale the recycling of ammonium and soluble reactive phosphorus, by a factor 6.5 and 4.6, respectively. Nizzoli *et al.* (2006) demonstrated that clams increase also denitrification rates, but to a much lower extent as compared to inorganic nitrogen recycling, as reported by Stief (2013). From then onwards, clams farming was considered non only something to protect from dystropohic outbreaks, but also a contributory cause of the environmental problems within the lagoon.

Melià *et al.* (2003), by means of a combined experimental and modelling approach, analyzed the risk of anoxia associated to the increasing clam density. It was found that the probability to deplete oxygen in the water column was very high in summer if clams density exceeded 500 ind m^{-2}. For the first time, clams themselves were considered as possible determinants of anoxia, due to their density, to the surface cultivated over the total and to their metabolism. Results from Melià *et al.* (2003) were shared with the community of fishermen in order to suggest sustainable densities in licensed areas and avoid the risk of anoxia and product loss.

A nutrient budget was also performed in an experimental site including a control and a cultivated area (Nizzoli *et al.*, 2007). Results suggest that the amount of N and P which is removed from the system with the clams biomass at the end of the farming cycle represents a very small amount if compared to that recycled to the water column via the bivalves excretion. This suggests that filter feeders do promote benthic-pelagic coupling and do favor the sedimentation of phytoplankton transported within the lagoon from either the freshwater or marine sides or produced *in situ*. In other words clams retain and concentrate phytoplankton within the lagoon, avoiding its transport to the open sea, and favor its conversion into dissolved solutes via excretion and coupled biodeposition and mineralization. Filter feeders therefore act as biological elements that remove particulate matter, retain a small fraction of what they filter in their biomass while produce large

biodeposition of faeces and pseuofaeces. Direct excretion and stimulation of microbial mineralization by labile organic input result in large and balanced sediment-water fluxes of inorganic nutrients.

Bartoli *et al.* (2003) performed a simple experiment to analyze whether clams may promote macroalgal growth via phytoplankton removal and nutrient mobilization. *Ulva* thalli were grown under controlled conditions above sediment with and without *T. philippinarum*. Benthic fluxes of oxygen and nutrients were measured together with the algae net growth rates. Results from this experiment clearly demonstrated that *Ulva* was growing at significantly higher rates in the presence of clams, likely due to a combination of higher water transparency and higher supply of nutrients from sediments.

After nearly three decades of investigation it appears clear that the Sacca di Goro, besides suffering from nutrient and organic matter loads delivered by the Po River has to face relevant ecosystem modifications due to its conversion into a mollusk farm. Our recent studies have demonstrated that the presence of clams, in such densities and over such surfaces, deeply alter the benthic metabolism of the whole lagoon ecosystem. If eutrophic estuarine areas as the Sacca di Goro are naturally prone to hypoxia/anoxia, the establishment of clams farming has at least doubled the risk of oxygen depletion. Furthermore, the metabolic activity of clams and their harvesting system determine a generalized release of nutrients and pollutants stored in the sediment (Carafa *et al.*, 2007).

These statements stress that the high productivity of the Sacca di Goro is the result of a fragile equilibrium. The dry and hot summer of 2015, for example, was critical for the lagoon, with some 5,000 t of product that was lost due to algal blooms and anoxia. The farmers have created a biological buffer outside the lagoon, in deeper marine areas where young clams are maintained in case of anoxia or high temperature risk. They are also used to move large amounts of clams outside the lagoon when conditions become critical. *Ruditapes* is very robust and can tolerate such manipulations. Still, they are costly and time-consuming.

If additional areas will be exploited for the farming activity the fragile ecological equilibrium of this lagoon will be further threatened and more investment will be required for canal dredging, bottom reshaping, and management of the farmed stock, thus inducing a feedback loop/short circuit.

Conclusions

Since its introduction, the clam farming has been managed mainly with business as usual criteria, orienting the production to the maximum allowable income. This approach was possible until mid 1990s, thanks to the support of the regional and national administrations, that supported farmers with compensation for the crop loss. Following more restrictive economic policies, in the last years fisherman cooperatives have invested resources for managing the lagoon, e.g. for improving hydrodynamics, for cleaning up sediments contaminated by fecal organic matter, and for controlling macroalgal spreading.

Despite either local or international project have addressed the sustainable exploitation of the lagoon resources, a sustainable oriented farming has not yet established. One of the major research effort was performed from 2003 to 2006, within the European project DITTY aimed at implementing a scientific support system for the exploitation of southern European coastal lagoons (Aliaume *et al.*, 2007). A DSS prototype was finally operating, but only at a pilot scale, without finding a real scale application (Mocenni *et al.*, 2010).

However, this and other projects allowed to integrate ecological, economic and social issues identifying the major drivers of the lagoon ecosystem structure and processes.

Overall, environmental, economic and social issues are tightly interlinked and their balance is, in other terms, the sustainability of the whole system. Indeed, often conflicts arise among different groups of stakeholders, mainly at the local scale, with contrasting goals ranging from heavy exploitation to severe conservation. This small but complex ecosystem is also a bottleneck between the Po river watersheds and the the adjacent coastal areas. This is causing further problems for policy and decision making from local to whole watershed scales (Viaroli *et al.*, 2012)

In fact, along with internal constraints, e.g. the clam farming, the Sacca di Goro lagoon is also under the influence of the large Po river basin. Therefore management and decision-making are constrained at different administrative levels, from local to regional to the interregional (whole watershed) levels.

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Status of Manila Clam, *Ruditapes philippinarum*, culture and fisheries on the West coast of North America

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The Manila clam *Ruditapes philippinarum* was inadvertently introduced into the Northeast Pacific Coast in the early 1900's with seed shipments of Pacific oysters (*Crassostrea gigas*). The species was first detected on the east coast of Vancouver Island in 1930 and spread rapidly. It is now found from the Central Coast of British Columbia to California. Production from aquaculture and commercial fisheries is approximately 5938 tones from British Columbia, Washington and California.

Commercial fisheries for Manila clams began in Washington State in the 1940's and the 1960's in British Columbia. Aquaculture of Manila clams supplemented by hatchery produced seed began in 1972 in Washington State; clam aquaculture was permitted since 1981 in California and 1985 in British Columbia. Washington State is unique in that tidelands may be privately held and this has promoted the development of intertidal shellfish aquaculture. Tidelands are leased from the State and the Provincial governments in California and British Columbia respectively.

Aboriginal groups in British Columbia and Washington State have differential access to wild stocks of Manila clams. Under federal treaties of 1854 and 1855, Washington tribes reserved the right to harvest fish and shellfish at all usual grounds and stations. Recent court decisions have upheld the Puget Sound tribes' rights to up to 50 percent of the sustainable yield from natural shellfish beds which exclude aquaculture sites. In British Columbia there are 597 Aboriginal Commercial Fishery licenses which are held by various First Nations (aboriginal communities) which subsequently distribute them to members. British Columbia First Nations' traditional harvest for Food Social and Ceremonial purposes may occur coast-wide where areas are open for harvest. In both Canada and the USA aboriginal groups who previously had strong cultural ties to shellfish are exploring or becoming increasing involved in clam aquaculture.

Clam aquaculture in North America utilizes conventional techniques included the use of hatchery produced seed to augment natural recruitment and predator netting to protect seeded stocks. Most harvesting is conducted by hand raking although the adaption of mechanical digging machines is being used at certain sites in Washington State and tested in British Columbia.

Social conflicts with clam aquaculture are increasing within the Strait of Georgia (British Columbia) and Puget Sound (Washington State) where upland urbanization is increasing. Controversy includes the concerns over the use of intertidal areas for shellfish cultivation, and licensing of new farms. These include increasing opposition to the use of predation netting which is believed to cause negative effects on wildlife, benthic ecosystems, increasing mechanization in the marine environment and public use and enjoyment of the marine areas. Non-point source pollution in these areas as a result of population increase have resulted in downgraded water quality and increased restrictions on harvesting in certain areas. Depuration of clams harvested from areas with decreased water quality is practiced in British Columbia only. Social conflicts are one of the drivers of research examining ecosystem interactions of clam aquaculture. Recent research projects have examined the ecological impacts that clam aquaculture may have in the marine environment as well as the ecosystem services that it may provide.

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Mapping the stranger: genetic diversity of Manila clam in European coastal lagoons

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Abstract: Manila clam *Ruditapes philippinarum* – synonym *Venerupis philippinarum* (Adams and Reeve, 1850) is one of the most successful marine invaders worldwide and represents almost 20% of worldwide mollusc total production. Originally distributed in the Indo-Pacific region, the species was introduced for aquaculture and fisheries in North America, Polynesia, the US Virgin Islands, as well as in Atlantic and Mediterranean coasts of Europe. Due to the commercial value of the species, population genetics and stock composition are of primary importance both in natural and invaded environments. Moreover, molecular genetics can provide valuable data in invasion biology, as, for example, the structure of the exploited populations, the origin of source populations and the routes of invasions. Furthermore, molecular data might be useful for the tracking of seafood products, as requested by the European Commission for European seafood products (see EC laws n° 178/2002, 509/2006, 510/2006 and their improvements). Despite all these considerations genetic data are lacking, especially for introduced populations, and should be urgently provided. In a framework of an European network of researchers involved in Manila clam studies, clams were collected in coastal lagoons and estuaries in both Mediterranean (Northern Adriatic Sea, Italy) and Atlantic (Spain and Portugal) coasts of Europe. Both mitochondrial and nuclear molecular markers were applied to investigate the genetic structure of populations, specifically by the direct sequencing of *16S* rDNA gene fragments and by the genotyping of 7 microsatellite markers previously used in native populations. Results demonstrated the occurrence of multiple haplotypes for *16S* rDNA, even if one main haplotype was identified in the European analyzed populations. However, few sequences were available in Genbank to make comparisons with native populations. Concerning the microsatellite data, the genotyping of Manila clam populations showed low genetic diversity among them, considering the Fst values, and a lack of genetic structuring when analysed by Structure software. Both these data suggest the absence of geographic differentiation among the introduced populations of Manila clam, as a result of introgression due to translocations and other human activities. The levels of genetic diversity within introduced populations were comparable to those described from native regions, suggesting that multiple introduction events and mixed source populations can counterbalance the loss of genetic diversity caused by the founder effect. However, it must be remarked that microsatellites showed a strong occurrence of null alleles when tested by Micro-checker, as previously observed in other bivalves, suggesting that the problem is differently distributed among Manila clam populations or specific loci, thus microsatellites should be carefully checked before used in extensive population studies. In the near future, population

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genetics and stock identification of this species on a large scale should be assessed using new powerful markers such as those obtained by NGS (Next Generation Sequencing) methods.

In conclusion, this research represents the very first extensive genetic investigation on Manila clam's European populations, defining the levels of genetic diversity that will help to inform management practices.

Keywords: *Ruditapes philippinarum*, Europe, *16S*, microsatellites, genetic diversity

Manila clam *Ruditapes philippinarum* - synonym *Venerupis philippinarum* (Adams and Reeve, 1850) is one of the most successful marine invaders worldwide and represents almost 20% of worldwide mollusc total production. The species was introduced in Europe in the early 1970s in North European Atlantic and Mediterranean coastal waters for commercial cultivation, especially in England and France (Gosling, 2003; Bald *et al.*, 2009). Then, from an English hatchery it was introduced in Northern Adriatic Sea in 1983 (Breber, 1985; Pellizzato *et al.*, 1989). As for Portugal, the first introduction is not documented, but it is believed that its introduction dates back to 1984, when it was reported at Ria Formosa. Since then, it was then reported in many estuarine systems all over the country (Gaspar, 2010). Up to know, Italy is the still the leading European country for Manila clam production, whilst China represents the first producer of Asari (Manila) clam in the world.

Despite its commercial value, it is almost surprising that genetic diversity of Manila clam was poorly investigated. As recently reported by Astorga (2014), even if Manila clam is listed as the first species among bivalves for estimated commercial values (FAO, 2013), the existing genetic information in literature is limited. Molecular genetics can provide valuable data (Chiesa *et al.* 2014a) being useful for tracing and tracking seafood products. Genetic profiles of cultivated and harvested clams can certify their geographic origin, preventing illegal selling and labeling (Kim *et al.*, 2014), guarantying consumer's safety and product's quality control (Filonzi *et al.*, 2010; Caldelli *et al.*, 2014), with both economic and social benefits.

However, few papers have been published concerning the genetic diversity and structure of Manila clam populations in its native range of distribution (see as examples Sekine *et al.*, 2006; Vargas *et al.*, 2008; Liu *et al.*, 2007; Mao *et al.*, 2011; An *et al.*, 2012; Kitada *et al.*, 2013; Nie *et al.*, 2015a), and in introduced ecosystems (Chiesa *et al.*, 2011; 2014a,b; 2016; Mura *et al.*, 2012; Hurtado *et al.*, 2012).

The present paper will summarize the data obtained on the genetic structure of introduced populations in Portugal, Spain and Italy, investigated by the direct sequencing of *16S* rDNA gene fragments and by the genotyping of 7 microsatellite markers.

Methods

Sampling procedures: Manila clam specimens were collected from both Mediterranean and Atlantic introduced populations, particularly in Italy, Portugal and Spain. See Table 1 for details.

DNA extraction and purification: High molecular

Table 1. Manila clam sampling sites in Portugal, Italy and Spain. The locations and the number of analyzed samples and for each site are provided

Country	Location	N (individuals)
Portugal	Ria de Aveiro Lagoon	24
Spain	Galician Coast	14
Italy	Venice Lagoon	35
Italy	Marano Lagoon	30
Italy	Po River Delta	33

weight genomic DNAs were extracted and purified through Wizard genomic DNA Purification kit (Promega). Ethanol-fixed mantle and foot tissue stored at -20°C were selected for the extraction, to avoid the interferences of the mtDNA "Doubly Uniparental Inheritance" (DUI) (Plazzi and Passamonti 2010). DNA extraction details are reported in Chiesa *et al.* (2011; 2014a).

16S rDNA analysis: Amplification of a *16S* rRNA gene fragment was achieved using primers *16S*ar-ALT (5' GCCTGTTTATCAAAAACATSG 3' and *16S*br-ALT (5' CCGGTCTGAACTCAGATCATGT 3') designed for Veneridae family (Mikkelsen *et al.*, 2006). Reaction details and PCR - touch down profile are described in Chiesa *et al.* (2011; 2014a). Fragments sequencing was performed by MACROGEN Europe service (Amsterdam, the Netherlands). The obtained sequences were compared with those available in genomic databases using Blast and multiple alignments of sense and antisense sequences were conducted using software as Clustal X and Sequencer 4.2 (Gene Code Corporation). Original sequences were aligned and compared with those downloaded from GenBank for *R. philippinarum*, *R. decussatus*, *R. variegatus* and *R. bruguieri* (see Chiesa *et al.*, 2014a for details). As for outgroups, two were selected: *Glauconome rugosa* and *Corbicula fluminea* (see Chiesa *et al.*, 2014a for details).

Phylogeographic analysis was performed through the analysis of aligned fragments of the *16S rDNA*. Haplotype network was performed in TCS v1.21 (Clement *et al.*, 2000), with confidence threshold at 95% for *Ruditapes* genus sequences (Hart and Sunday, 2007; Lucentini *et al.*, 2011). A median-joining network (Bandelt *et al.*, 1999) was constructed using Network 4.611 for *R. philippinarum* haplotypes and outgroups. (see Chiesa *et al.*, 2014a for details).

The identification of variable and parsimony informative sites, the translation of nucleotide sequences, the pairwise genetic distances, the nucleotide base composition and the transition/transversion ratios were calculated using MEGA 6.06 (Tamura *et al.*, 2013).

Spatial or demographic expansion was estimated through Tajima's D neutrality test (Tajima 1989). Statistical selection of best-fit models of nucleotide substitution was performed by means of jmodeltest (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). On the basis of these results, the Jukes-Cantor model was used to assess the evolutionary history among *R. philippinarum*, *R. decussatus* and other outgroups; Maximum Likelihood and Neighbour Joining methods were inferred in MEGA6.06 estimating standard error by a bootstrap procedure (1,000 replicates). See Chiesa *et al.*, 2014a for details on statistical analyses.

Microsatellites analyses: All samples were genotyped for seven microsatellite loci previously used in different studies with Manila clam: *Asari16*, *Asari23*, *Asari24*, *Asari54* (Yasuda *et al.*, 2007), and *Ktp5*, *Ktp8*, *Ktp22* (An *et al.*, 2009), chosen according to their variability among different samples. Polymerase chain reaction (PCR) and microsatellite collection were performed as described in Chiesa *et al.* (2011). The quality of the seven microsatellite markers was tested as suggested by Vignaud *et al.* (2013): Micro-checker v2.2.3 (van Oosterhout *et al.*, 2004) was used to check genotyping errors and null alleles potentially present. Linkage Disequilibrium between each pair of loci was tested though GenePop (Rousset, 2008).

To evaluate allelic richness, private alleles and test for the Hardy-Weinberg equilibrium for each locus both in the entire dataset and in each pool (Adriatic or Atlantic), the software GenAlEx 6.4.1 (Peakall and Smouse, 2006) was used. Since the presence of null alleles is a compromising-factor in the evaluation of genetic distances, the ENA (Excluding Null Alleles) method implemented in FreeNA (Chapuis and Estoup, 2007; http://www.montpellier.inra.fr/URLB/) was performed and Fst values were computed using both corrected and uncorrected data. Correlation analysis and both t-test and F-test were performed to evaluate the correlation between corrected and observed frequencies. (See Chiesa *et al.*, 2016 for details). Furthermore, to investigate whether the presence of null alleles influenced the allelic diversity, a per locus dispersion graph and a correlation analysis were performed. (See Chiesa *et al.*, 2016 for details).

Using Structure 2.3.3 program (Pritchard *et al.*, 2000) a Bayesian approach was performed with the aim to estimate the a posteriori probability of a given number of stocks. To detect the K-value that best fit the data, both approaches were used: the mean posterior probability of the data [Ln(K)] and the Evanno *et al.* (2005) method of delta K using STRUCTURE HARVESTER (Earl and VonHoldt, 2012). Structure assignment test was performed for the whole dataset or removing all samples affected by null alleles at three or more loci.

Results

mtDNA: The *16*SrDNA sequences were successfully sequenced for 133 individuals of *R. philippinarum* with a final alignment of 438 bp (see Chiesa *et al.*, 2014a). Twenty haplotypes were identified including outgroups, clustering into 5 separate haplogroups. One haplogroup included 12 haplotypes for *R. philippinarum* (Chiesa *et al.*, 2014a). The original Italian, Spanish and Portuguese samples (GenBank A.N. KF736199-211) were included within haplotypes Rphap1-10 and Rphap12, whilst Rphap11 contained only a Chinese sequence (GenBank A.N. DQ356383) (Chiesa *et al.*, 2014a). Rphap1 (weight =0.174) had the biggest haplotype probability among *R. philippinarum* haplotypes; other haplotypes were rare (weight ranged from 0.0006 to 0.162). (Chiesa *et al.*, 2014a). The Tajima's Neutrality Test performed on all *R. philippinarum* sequences showed the occurrence of 12 segregating sites (S) and a significant ($p<0.05$), Tajima statistics test ($D = -1.914$). (Chiesa *et al.*, 2014a).

If considering only European sequences, S was 10 and Tajima statistics test D was -1.789 ($p<0.05$). Considering only Adriatic or Atlantic samples, the values changed as S was 6, whether D was -1.755 for Adriatic samples and for Atlantic samples D -1.487 for Atlantic ones, both with $p>0.05$. (See Chiesa *et al.*, 2014a for details). J modeltest identified JC as the best model. Bootstrap NJ and ML phenograms performed with this model showed almost the same topology, with a clear separation of *R. philippinarum* from congeneric species. A significant node separation (99/100 bootstrap) was observed within *R. philippinarum* haplotypes, separating the Chinese haplotype Rphap11 from all the European haplotypes (Chiesa *et al.*, 2014a).

The haplotype Rp hap1 was the most frequent, commonly distributed among all populations. On the opposite, the other 10 haplotypes were rare and locally distributed, showing a specific geographic structuring (Chiesa *et al.*, 2014a).

Microsatellites: As for microsatellites, all loci were affected by missing amplifications. At the locus *Asari16*, 5% of samples did not amplify. The percentage was higher at the other loci: 25%, 27% and 60% for loci *Asari23*, *Asari24*, and *Asari54* respectively; 54%, 35%, 45% for loci *Ktp5*, *Ktp8*, *Ktp22*, respectively. Considering the geographic distribution, the occurrence of missing amplifications ranged from 27% in the Venice population to 49% in Marano population, 29% within the Spanish population, 39% for Delta Po and 43% for the Ria de Aveiro populations. These observations are in accordance with Micro-checker results and FreeNA tests, showing that each locus could be globally affected by null-alleles, except for locus *Asari16* (Chiesa *et al.*, 2016). In the Marano lagoon population, high frequency of missing amplifications resulted in data deficiency of Micro-checker tests (Chiesa *et al.*, 2016). The comparison of gene frequencies corrected by means of FreeNA with original ones, underlined a correlation of up to 80% and t-student and F tests showed no statistical significant differences between these two series. The graph and the correlation analysis performed to investigate whether the presence of null alleles influenced the allelic diversity indicated differences among loci (Chiesa *et al.*, 2016).

Exact test for genotypic Linkage Disequilibrium was significant at 0.05 level for 17 out of 21 comparisons. The same LD test was performed for each couple of loci, removing samples affected by null alleles: results underlined the absence of linkage among different loci.

The expected heterozygosity (He) across populations differed among the seven analyzed loci ranging from 0.47 ± 0.08 for *Asari54* to 0.85 ± 0.02 for *Ktp22*; the observed values (Ho) of heterozygosity ranged between 0.23 ± 0.05 for *Ktp5* and 0.82 ± 0.04 for *Asari16*. (See Chiesa *et al.*, 2016 for details).

Per population, F ranged from -0.02 (Po Delta

population locus *Ktp8*) to 0.93 (population Po Delta locus *Ktp22)* (See Chiesa *et al.*, 2016 for details); Hardy-Weinberg (HW) analysis results underlined that *Asari16* was under equilibrium, in contrast the others were in equilibrium, not under equilibrium or monomorphic, showing variations across populations. HW results across populations underlined that only *Asari 16* was under equilibrium, and the remaining loci were not (Chiesa *et al.*, 2016).

Fst values calculated with or without ENA corrections did not change significantly (Chiesa *et al.*, 2016). Exploratory structure runs demonstrated that a burn-in period of 100,000 steps, followed by 500,000 steps of data collection, for each of the combined conditions mentioned in materials and methods, ensure the convergence of the Monte Carlo Markov Chain (MCMC). When estimating K, a discrepancy emerged between Pr (X/K), corresponding to LnP(D), and ΔK; the highest LnP(D) was found for K= 5 but ΔK clearly indicated the uppermost K=2 (Fig. 1). It was demanding to choose a single K to performed a structure's cluster, in fact if Evanno *et al.* (2005) found that LnP(D) might fail, is also true that Evanno's method might underestimate K (Waples and Gaggiotti, 2006). Therefore, the structure's bar plot was performed both with K=2 and K=5 (Fig. 2). In both cases, a geographical pattern was lacking; for K=2 almost all individuals were assigned to both genetic clusters, analogously, for K=5 almost all individuals were assigned to three or more clusters (Fig. 2). Genetic structure performed removing the entire Marano population, or removing all samples affected by null alleles at three or more loci, presented the same results of the global pool.

Discussion

Concerning mtDNA analyses, the direct sequencing of a *16S*rDNA fragment showed interesting results: European introduced populations were characterized by one common haplotype, Rphap1, whilst the other 10 haplotypes were rare and represented by only 1 or 2 sequences (Chiesa *et al.*, 2014a). When comparing the two macro areas Atlantic vs Adriatic, haplotype frequencies and the haplotype minimum spanning tree underlined a biogeographic differentiation among regions (Chiesa *et al.*, 2014a). Apart from the common haplotype Rphap1, the other haplotypes differently occurred depending on the sampling sites. Haplotype Rphap1 could represent an haplotype characteristic of the ancestor lineage introduced in Italy, Spain and Portugal (Chiesa *et al.*, 2014a). However, the occurrence of rare haplotypes with a specific geographic distribution suggested multiple founder effects (Chiesa *et al.*, 2014a).

As for microsatellite markers, they seemed to be useful and promising to characterize fisheries and aquaculture Manila clam stocks (Chiesa *et al.*, 2011; An *et al.*, 2012; Kitada *et al.*, 2013; Nie *et al.*, 2014; 2015a,b). However, our investigation clearly showed that missing amplifications and null alleles are

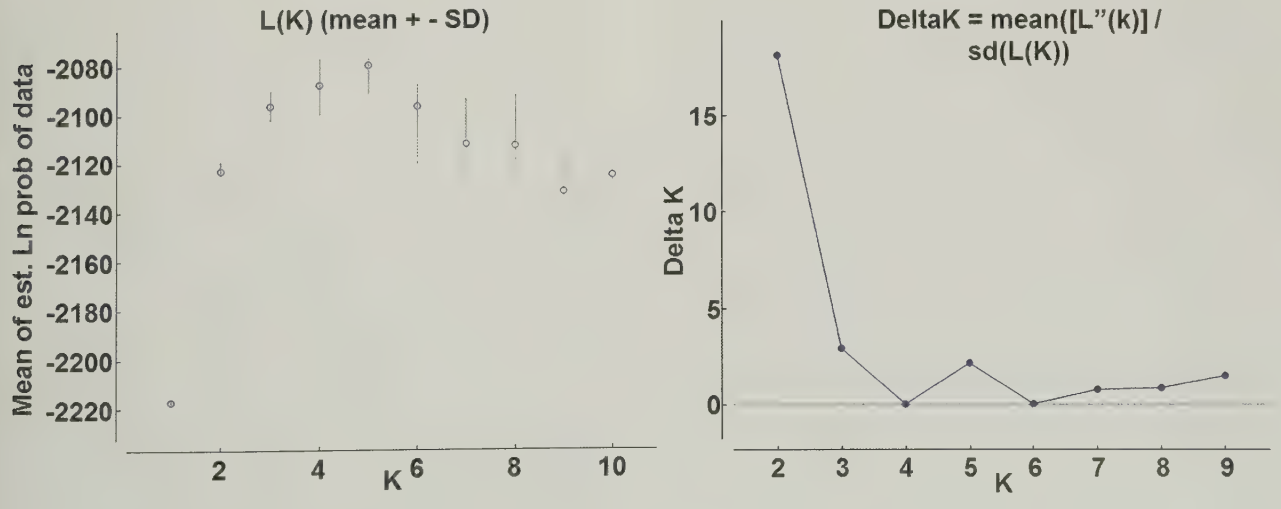

Fig. 1 Inference of true K. a. Use of Ln(K); b. Use of an ad hoc quantity [ΔK].

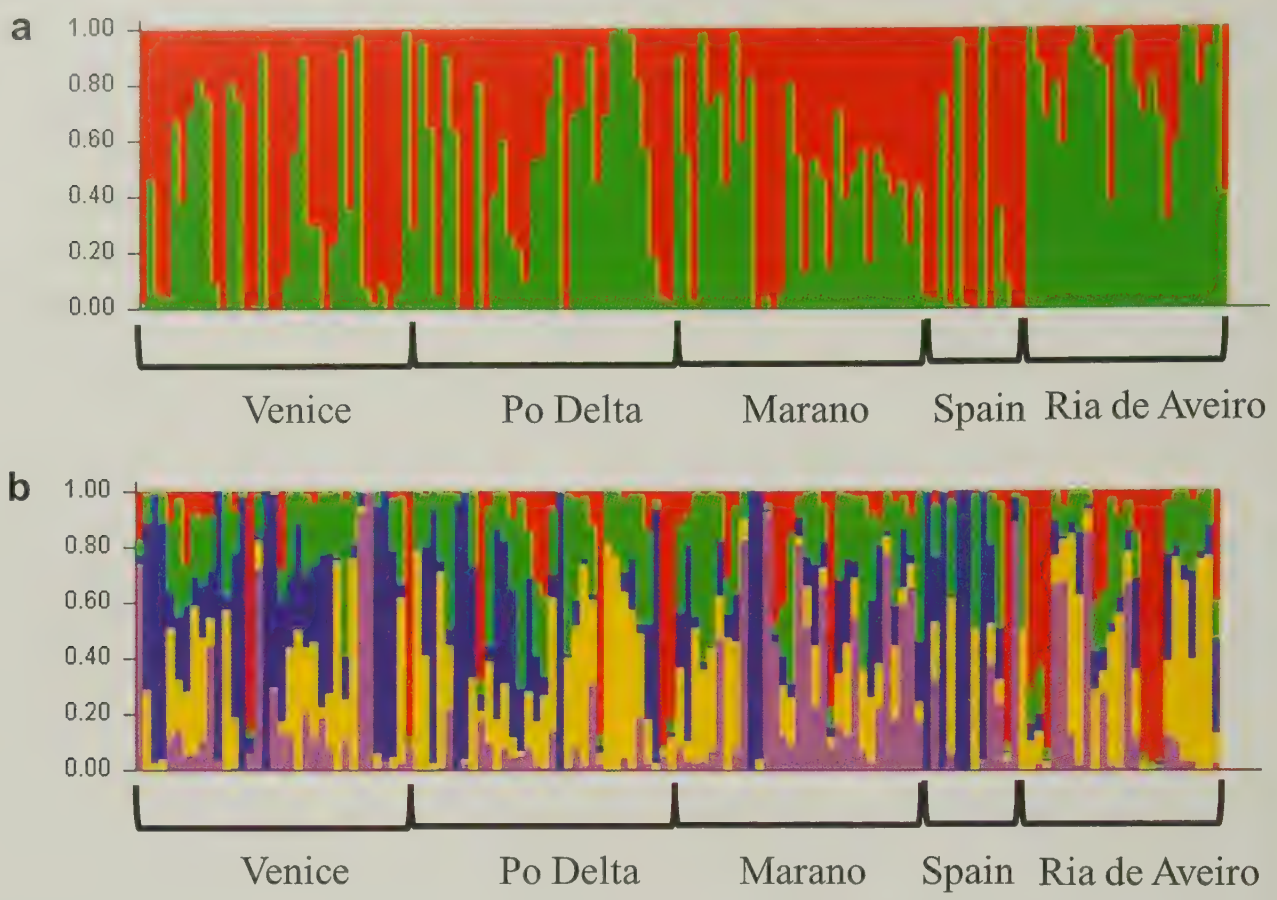

Fig. 2 Genetic structure revealed by 7 microsatellite markers in *R. philippinarum* for (a) K=2 and (b) K=5. Each vertical bar represents a single individual divided into color segments, proportional to its membership within the K genetic clusters.

critical points in this species genotyping, especially when comparing different populations. The results herein presented showed both the presence of missing amplifications in the Manila clam populations herein analyzed, and null alleles in six of the seven investigated loci, previously used in other Manila clam studies for stock analyses and conservation genetics. The occurrence of null alleles is very common in animal populations, especially in molluscs (see as examples Arias-Pérez *et al.*, 2012; Marin *et al.*, 2012). Null alleles were likely to be encountered especially in populations with a large effective size, with an unusually high mutation rate in the flanking regions, and that have diverged from the population from which the cloned allele state was drawn and the primers designed (Chapuis and Estoup, 2007). To date, several statistical approaches were reported for correcting allele frequencies when null alleles are occurring (van Oosterhout *et al.* 2004; Chapuis and Estoup 2007; Wagner *et al.*, 2006). These methods can be reliable to evaluate levels of population differentiation, as Fst, but they are not useful when performing assignment tests. As reported by Carlsson (2008) "they rely on accurate information about the multilocus genotype of individuals" . Furthermore, the frequencies correction seems to be particularly powerful when null alleles are related to an incorrect scoring of a/anull genotypes as an homozigote a/a. Unfortunately, our data showed an anull/anull pattern in several cases, and it is difficult to understand the feasibility of frequencies correction methods in this condition (Chiesa *et al.*, 2016). It is remarkable that, even though in our data several loci were affected by null alleles showing an anull/anull pattern, globally the number of observed alleles was comparable to those observed in other populations. Exact test for genotypic Linkage Disequilibrium, were non significant for all

comparisons when removing samples affected by null alleles, showing that the statistical values were reinforced. The influence of null alleles on Linkage Disequilibrium was already demonstrated (Sved *et al.*, 2013).

The mean values of observed heterozygosity (Ho) were in agreement to those reported for *Asari16* in other Manila clam populations by Yasuda *et al.* (2007), Mura *et al.* (2012) and Kitada *et al.* (2013), but lower for other loci (see Yasuda *et al.*, 2007; An *et al.*, 2012 for comparisons).

Fst values calculated with or without ENA corrections did not change significantly (Chiesa *et al.*, 2016). These data underlined a very low genetic pairwise differentiation of the investigated populations (Fst < 0.05) (Chiesa *et al.*, 2016).

Finally, concerning the structure analysis, two main issues must be highlighted. First, it is interesting that the discordance between reliable K values suggested by LnP(D) (K=2) and ΔK (K=5). Such discordance might be related to the presence of null alleles; as also reported in the Structure's manual "A serious problem arises when data are missing in a systematic manner, as with null alleles" . Only few discordant information are available about the effects of null alleles on genetic assignment testing, even if Carlsson (2008) clearly underlined that null alleles cause a reduction in the power to correctly assign individuals.

Conclusions

Biogeographic and phylogenetic analyses obtained by *16S*rDNA sequencing showed a complex scenario for Manila clam introduced populations, dominated by multiple introductions of individuals coming from different sources (Chiesa *et al.*, 2014a). Microsatellite genotyping of Manila clam populations introduced in Italy, Spain and Portugal showed low genetic diversity among them, considering the Fst values, and a lack of genetic structuring when analysed by Structure (see Fig. 2). Both these data suggest the absence of genetic differentiation among the introduced populations of Manila clam, as a result of introgression due to translocations and other human activities. This conclusion is in agreement with those obtained by *16S* rDNA analyses (see Chiesa *et al.*, 2014a).

Although, the results herein reported showed a strong occurrence of null alleles; even thought evidences of null alleles emerged in previous studies on Manila clam (Chiesa *et al.* 2011a; Mura *et al.* 2012; An *et al.* 2012, Kitada *et al.* 2013; Nie *et al.* 2014) where Micro-checker/FreeNA tests were largely missing, or used only to confirm their occurrence (An *et al.*, 2012). The present work underlined not only the diffuse presence of null alleles, but particularly suggested that such problem is differently distributed among populations or loci (Chiesa *et al.*, 2016).

The authors cannot exclude that a limited sampling session may have affected the results here presented, both in terms of genetic structuring and of null alleles influence. Nevertheless, as already suggested by Kelly *et al.* (2011), genotyping errors should be reported to increase awareness of problematic markers in the scientific community, especially when deviations from HWE are detected (DeWoody *et al.*, 2006). This is particularly important, and generally applicable to all studies that employ microsatellites, independently from the taxon involved, to allow a careful selection of microsatellite loci before using them for population genetics studies (Kelly *et al.*, 2011).

Considering that microsatellites are both money- and time- consuming, an accurate selection of the genetic markers might be fundamental. Case by case, a reliable panel of loci should be selected before being applied to population genetics studies. However, few papers underlying genotyping errors are encouraged to be published by scientific journals. In cases where microsatellites seemed to be problematic, the possibility to use other markers should also be considered for stock identification and population genetics.

MtDNA sequence analysis – previously used in other valuable species of conservation or aquaculture interest (Chiesa *et al.*, 2014c; Lucentini *et al.*, 2011; 2014) – or new powerful NGS (Next Generation Sequencing) derived SNPs (Single Nucleotide Polymorphisms- see for example Kim *et al.*, 2014) can be alternative solutions. Particularly SNPs could supply new genetic informations based on a wider dataset, hopefully less affected by technical problems as

microsatellites, meanwhile being highly informative.

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An a-priori evaluation model for restoration of fisheries population of the Manila clam and a countermeasure example in Ise Bay

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Abstract: The national production of the Manila clam, *Ruditapes philippinarum*, fishery has been continuously declining since the mid 1980s in Japan. The total production of asari in Ise Bay exceeded 10,000 tons per year in 1980's. However, it started to decrease in the mid 1990s, and it has been less than 5,000 tons. Fisheries Agency of Japan has been implementing stock enhancement programs, including fisheries management, fishing ground development and reseeding as countermeasures for the production decline. In order to attain successful stock enhancement, it is first of all necessary to elucidate the causes of stock depletion in each stage in life cycle of the clam. Then, one must make a plan to remove or reduce the effects of the elucidated causes taking into account what measures should be applied to what location to what extent. In the present study, we introduce about two topics. First topic is about the development of an a-priori evaluation model for restoration of the asari clam in Ise Bay. And the second topic is an example of countermeasure for asari stock recovery in Ise Bay. In Ise Bay, asari juveniles often do not survive until adult stage. So we have developed an effective juvenile collection method for transplantation and we are trying to develop a nursery ground for high growth and low mortality of asari.

Key words: Manila clam, *Ruditapes philippinarum*, asari, Ise Bay, a-priori evaluation model, nursery ground, spawning ground

Asari is one of popular seafood in Japan: however, the fishery production of asari is decreasing and has almost no sign of restoration in Japan. The production of asari in Ise Bay has also been declining, which was around 10,000 t per year from 1980 to 1995 and then sharply dropped to the current level of less than 5,000 t per year for a decade (Fig. 1).

Water currents transport the eggs and larvae of asari for several weeks. The larvae must reach an appropriate habitat to settle on the bottom and start the benthic life. The amount of spawned eggs and arrival of larvae to fishing ground are important factors determining the new recruitment level. For the benthic stage after the settlement, parameters such as food availability, dissolved oxygen (DO) level, predation, wave conditions and fishing intensity are important to determine the stock level. For

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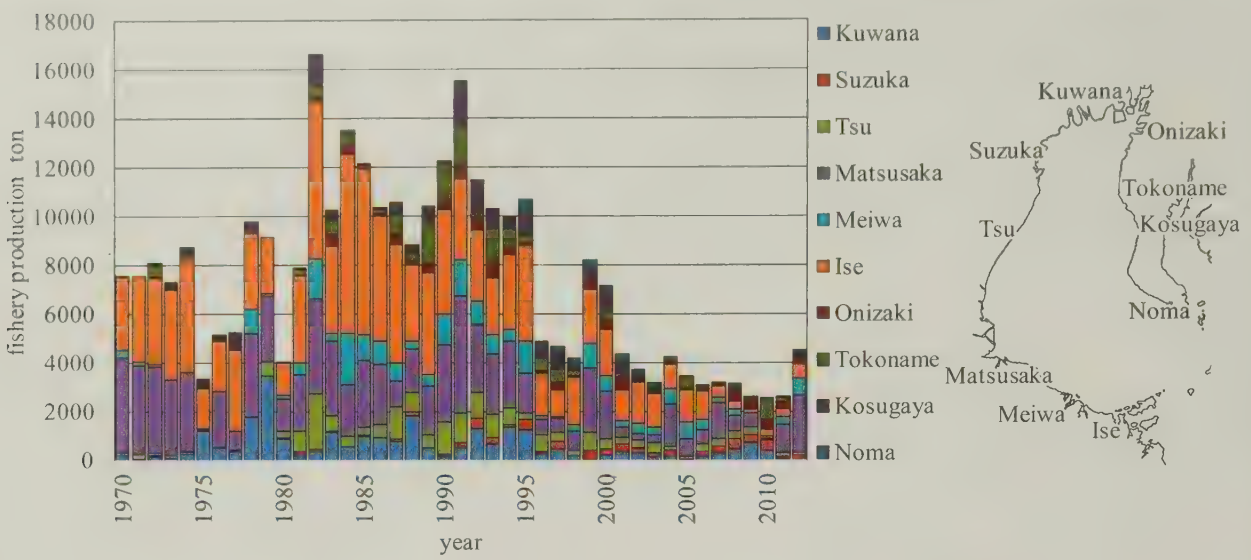

Fig. 1. The trend in annual production of asari in Ise Bay

each fishing ground, we need to identify causes of population decline and design countermeasures. We have to decide what operations should be done at what size at what location to effectively restore fishery population of asari.

This report describes two topics. The first topic is about the development of an a-priori evaluation model for restoration of the asari clam in Ise Bay. This model quantitatively determines the effects of size and location of spawning ground and nursery ground on the recruitment, taking into consideration the life cycle characteristics of asari. The second topic is an example of countermeasure for asari stock recovery in Ise Bay. In Ise Bay, asari juveniles occur at high density in many locations, but they often do not survive until adult stage. Therefore, we have developed an effective juvenile collection method for transplantation to a nursery ground for higher growth and lower mortality of asari.

An a-priori evaluation model for restoration of the asari clam

Configuration of model components: The outline of the model components is shown in Fig. 2. This model consists of 5 parts dealing with two life stages of asari. First of all, we assume that one adult clam larger than 20 mm in shell length produces 500,000 eggs, and we estimate the total number of eggs produced in each fishing ground based on this value. Spawning of asari occurs in spring and autumn in Ise bay.

To evaluate the amount of larval transportation, we calculate the connectivity among fisheries grounds based on the hydrodynamic simulation in Ise Bay during the 15-day planktonic stage of asari. Then, we calculate the number of larval settlement in each fishing ground.

We then calculate the mortality and the growth of the settled asari based on our previous field studies. We give different mortality rate for each fishing ground depending on the disturbance of the sea bottom by wave action. One of the conditions for mortality estimate is that asari cannot live in areas deeper than 4 m because of hypoxia. Finally, we obtain the number of adult asari larger than 20 mm in shell length, and the next life cycle is followed with the calculated values as the initial conditions.

Transportation of planktonic larvae of asari: Planktonic larvae of asari are transported passively by hydrodynamic force. We assumed that hydrodynamic force consists of 3 factors: wind driven force, river discharge and ocean current. We performed a principal component analysis and cluster analysis of the hydrodynamic data of Ise Bay from 1983 to 2003 to classify them into groups. We found that the hydrodynamic characteristics could

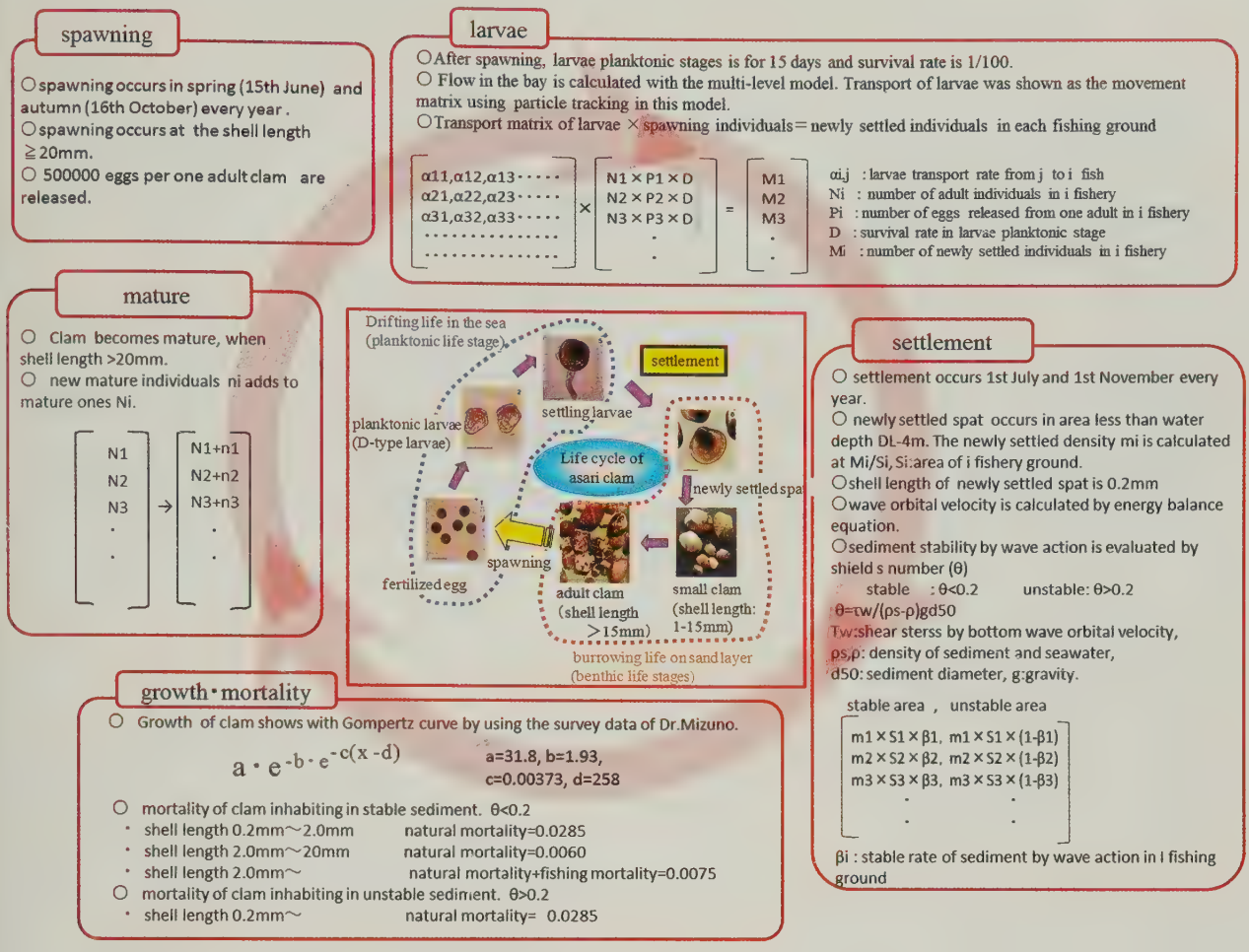
spawning
○spawning occurs in spring (15th June) and autumn (16th October) every year.
○spawning occurs at the shell length ≧20mm.
○ 500000 eggs per one adult clam are released.
larvae
○After spawning, larvae planktonic stages is for 15 days and survival rate is 1/100.
○ Flow in the bay is calculated with the multi-level model. Transport of larvae was shown as the movement matrix using particle tracking in this model.
○Transport matrix of larvae × spawning individuals = newly settled individuals in each fishing ground
αi,j : larvae transport rate from j to i fish
Ni : number of adult individuals in i fishery
Pi : number of eggs released from one adult in i fishery
D : survival rate in larvae planktonic stage
Mi : number of newly settled individuals in i fishery
mature
○ Clam becomes mature, when shell length >20mm.
○ new mature individuals ni adds to mature ones Ni.
Drifting life in the sea (planktonic life stage)
settlement
settling larvae
planktonic larvae (D-type larvae)
Life cycle of asari clam
newly settled spat
spawning
fertilized egg
adult clam (shell length >15mm)
small clam (shell length: 1-15mm)
burrowing life on sand layer (benthic life stages)
settlement
○ settlement occurs 1st July and 1st November every year.
○ newly settled spat occurs in area less than water depth DL-4m. The newly settled density mi is calculated at Mi/Si, Si:area of i fishery ground.
○shell length of newly settled spat is 0.2mm
○wave orbital velocity is calculated by energy balance equation.
○sediment stability by wave action is evaluated by shield s number (θ)
stable : θ<0.2 unstable: θ>0.2
θ=τw/(ρs-ρ)gd50
Tw:shear sterss by bottom wave orbital velocity,
ρs,ρ: density of sediment and seawater,
d50: sediment diameter, g:gravity.
stable area , unstable area
βi : stable rate of sediment by wave action in I fishing ground
growth・mortality
○ Growth of clam shows with Gompertz curve by using the survey data of Dr.Mizuno.
a=31.8, b=1.93, c=0.00373, d=258
○ mortality of clam inhabiting in stable sediment. θ<0.2
・ shell length 0.2mm～2.0mm natural mortality=0.0285
・ shell length 2.0mm～20mm natural mortality=0.0060
・ shell length 2.0mm～ natural mortality+fishing mortality=0.0075
○ mortality of clam inhabiting in unstable sediment. θ>0.2
・ shell length 0.2mm～ natural mortality= 0.0285

Fig. 2. An a-priori evaluation model for restoration of fisheries population of the Manila clam

be classified into 5 groups in spring and 6 groups in autumn. Years that belong to the same group have similar hydrodynamic pattern and thus similar transportation pattern of asari larvae.

The current in Ise Bay was calculated with the multi-level hydrodynamic model. The transportation of asari larvae was calculated with the particle tracking method concerning the vertical migration. Transportation of larvae of asari were expressed with matrixes from the calculated results of each groups. The model estimated that the larval transportation between Ise Bay and the adjacent Mikawa Bay is little, and the larval transportation is more active in spring than in autumn.

Mortality and growth of asari: The mortality and growth of asari were studied in our previous studies in Ise Bay. Growth pattern of asari can be described by Gompertz model. In Ise Bay, asari juveniles often fail to grow up to commercial size. One of the mortality causes of asari juveniles is the suspended load transport by wave action. Asari juveniles can be washed up to unfavorable places by strong waves and die. The Shields number from 0.2 to 0.5 causes the suspended load transport of asari juveniles. Therefore, in this model, the natural mortality of asari was expected to be high with the Shields number (θ) exceeding 0.2.

$$\theta = \tau_w / [(\rho_s - \rho) g D_{50}]$$

where τ_w is the bottom shear stress by water wave $=1/2\ \rho\ f_w u_{orb}^2$, u_{orb} is the bottom wave orbital velocity, f_w is friction factor $=\exp[5.213(k_s/A_{orb})^{0.194}]-5.977$, k_s is the hydraulic (Nikuradse) roughness length$=2.5D_{50}$, A_{orb} is the bottom wave amplitude$=u_{orb}T/2$, T is the wave period, D_{50} is the median grain size, ρ_s is sand density, ρ is the sea water density, and g is the gravitational acceleration.

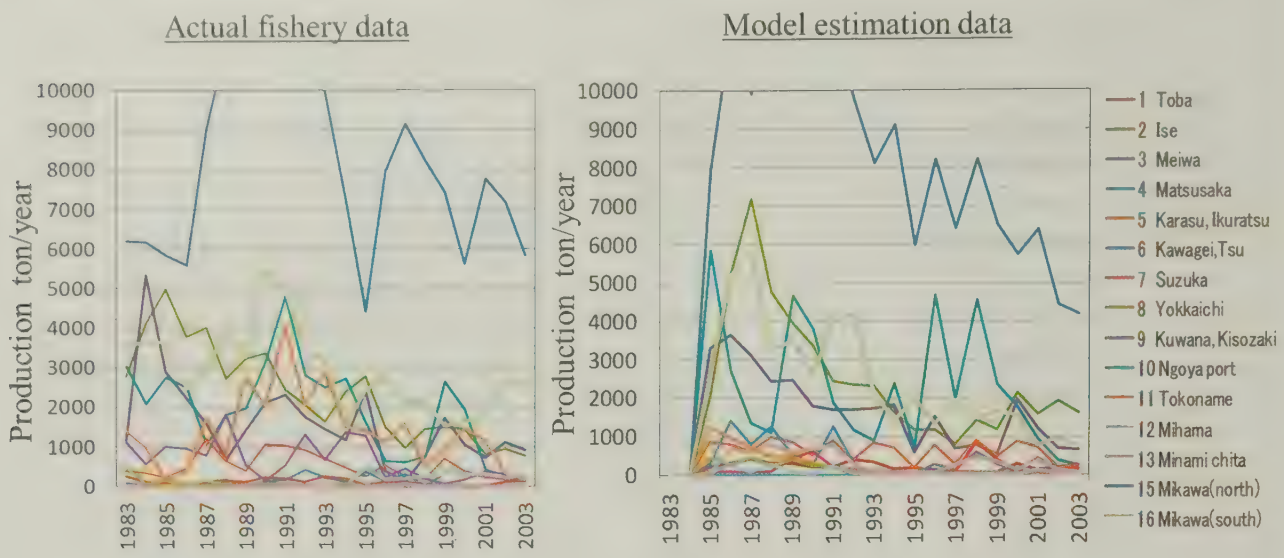

Fig. 3. The model estimations for the annual production of asari in Ise Bay

In Ise Bay, it takes about two years for asari to reach harvestable size of 20 mm; therefore, fishing mortality is applied to asari older than two years old on top of the natural mortality.

Model estimations for the annual production of asari in Ise Bay: The validity of this model is shown in Fig. 3. The left graph shows the actual annual production of asari in Ise Bay, and the right is the model estimations. Although there are some minor differences, the model estimations are roughly the same as the actual data.

Evaluation of a spawning ground or a nursery ground by an a-priori evaluation model: In order to increase asari production by more than 1000 tons by 2003, what should be the size and location of a spawning ground or a nursery ground developed in 1988? We want to derive answers for this kind of question using this model. Spawning grounds are to be developed in such a way that we can expect increased egg production and larval supply to the bay. Nursery grounds are to be designed to have stable sediment under strong wave environments to increase survival of asari juvenile.

The estimated increase of asari production by development of nursery or spawning grounds of variable scales is shown in Fig 4. The left figure shows the effect of nursery ground development and the right figure spawning ground development on the increase in asari production. Developing a nursery ground from region 3 to 6 is estimated to be effective, and spawning ground should be developed in region 7 and 11. In order to increase asari production by more than 1000 tons in 2003, we should develop a 100 ha nursery ground and spawning ground with 100 million spawners in these fishing grounds in 1988.

There are three large rivers flowing into Ise Bay, creating water current towards the bay mouth. Therefore, we suggest that spawning grounds (source) should be developed at inner part of Ise Bay and the nursery (sink) in western part of the bay to optimize the source-sink dynamics of larval supply and juvenile settlement.

Example of countermeasures for asari stock recovery in Ise Bay

Development of Collection device for asari juveniles: In Ise Bay, asari juveniles often fail to grow up to the recruitment size of 2 mm. Some of the causes of the juvenile mortality are disturbance of sediment by strong waves and river discharge after a storm event. For this, fishers in Matsusaka, Ise Bay collect juveniles for transplantation every year. They use a rake with fine mesh to collect juveniles and transplant them to places with better survival.

We have developed a device to help the fishers

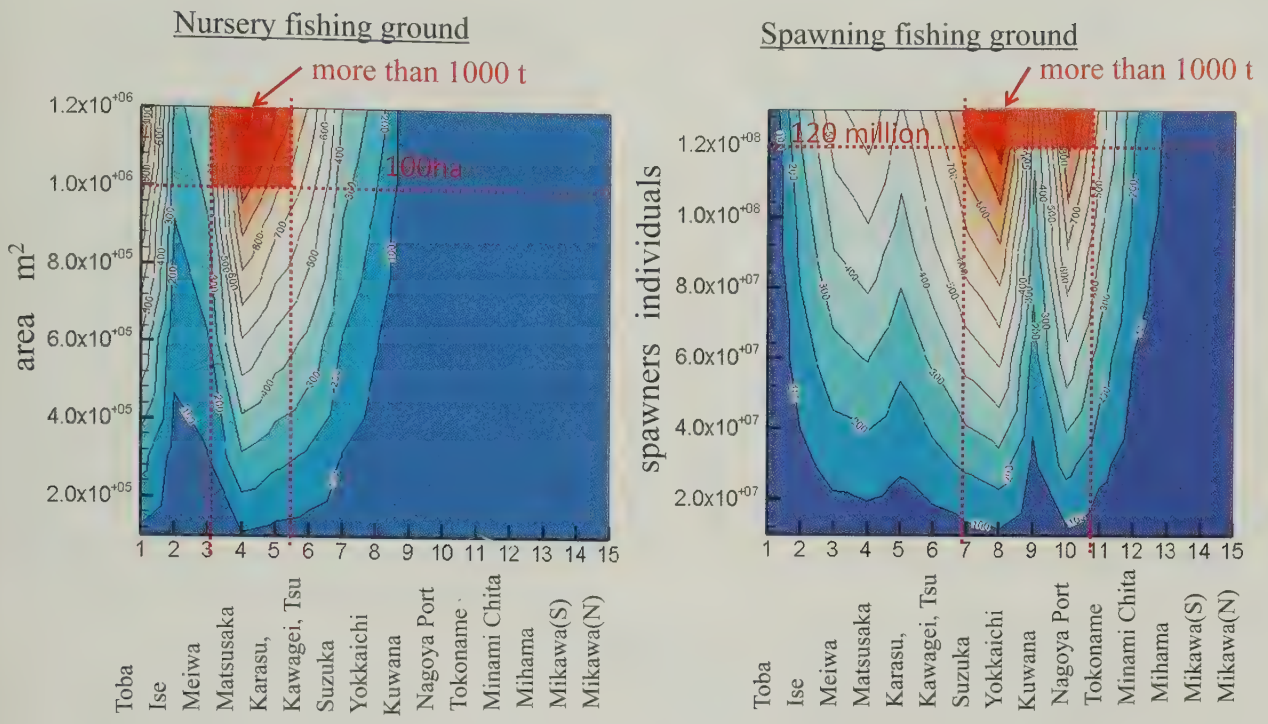

Fig. 4. estimated production increase of asari by development of nursery or spawning grounds of variable scales

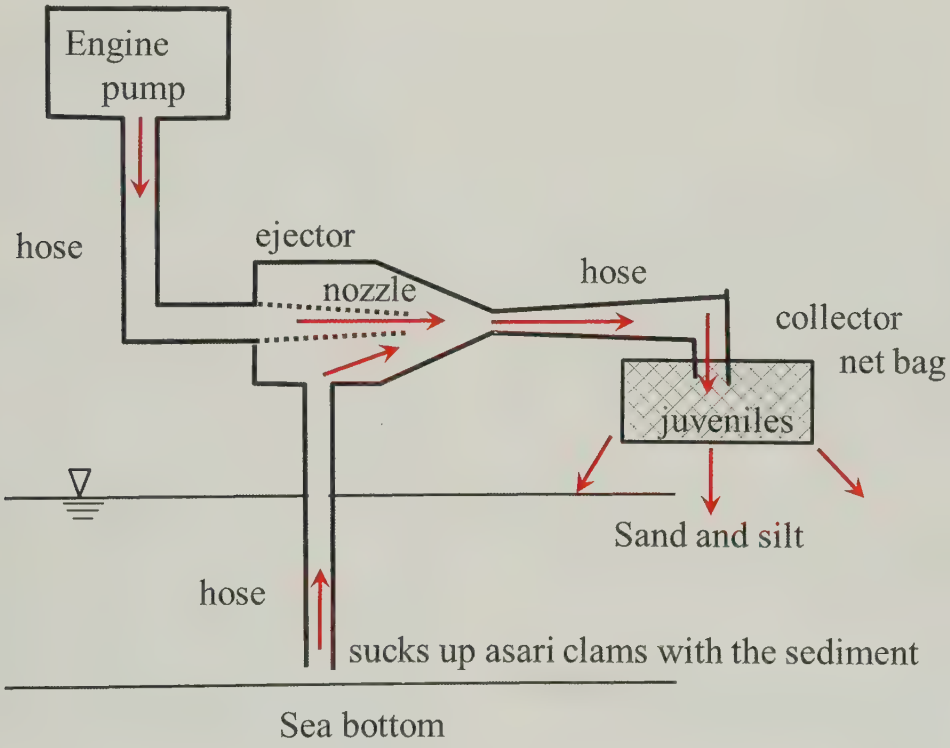

Fig. 5. Mechanism for collecting asari juveniles

collect asari juveniles more efficiently (Fig. 5). A strong current produced by the engine pump creates suction in the ejector, and this sucks up clams with the sediment. Sand and silt are washed through the net, leaving asari juveniles larger than 4 mm in shell length behind.

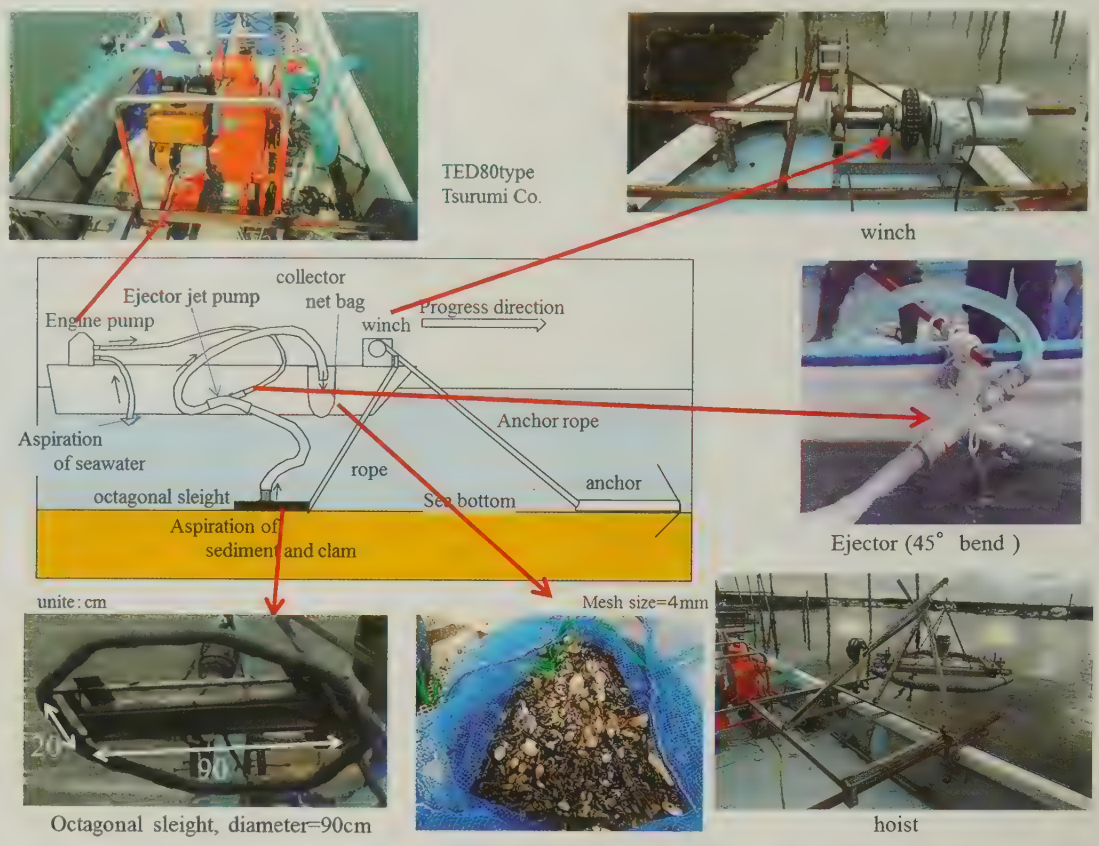

Fig. 6. Collection device of asari juveniles

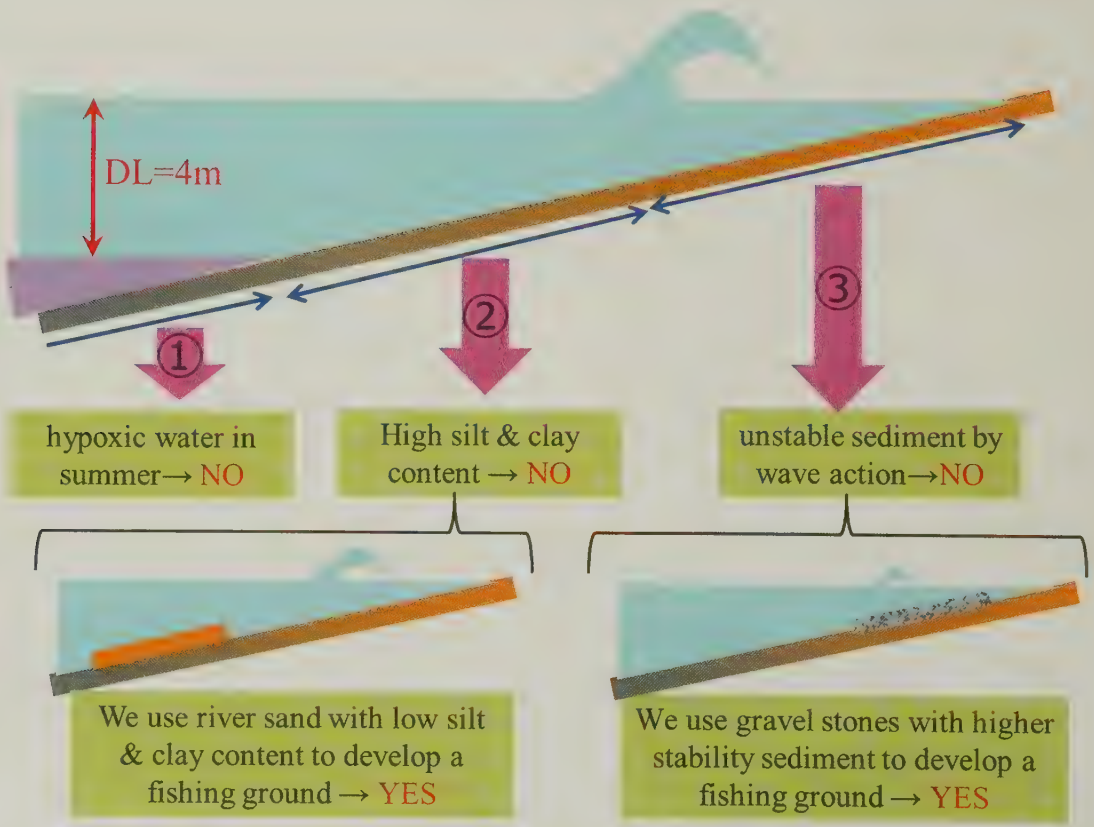
DL=4m
①
②
③
hypoxic water in summer→ NO
High silt & clay content → NO
unstable sediment by wave action→NO
We use river sand with low silt & clay content to develop a fishing ground → YES
We use gravel stones with higher stability sediment to develop a fishing ground → YES

Fig. 7. Concept for fishing ground development of asari

It takes about 1 hour to install the collection device to a boat (Fig. 6). For the operation, we at first anchor the boat and go astern for about 100 m. Start the asari collection device and slowly winch up the anchor rope as we collect asari. We transfer the collected asari to another boat for transportation and releasing. In this example, 5 people worked for 4 days to transplant about 4 million individuals of asari juveniles.

Fig. 8. Development of two sediment types of asari fishing grounds

Development of effective asari fishing ground: The concepts for asari fishing ground development is shown in Fig. 7. Ise Bay has a problem of anoxic water. So a fishing ground must be developed in areas shallower than 4 m where anoxia does not occur. We use river sand with low silt and clay content to develop a fishing ground because fine particle sediments are not suitable for asari habitat. It is important to stabilize the sediment by using gravel stones in areas with strong waves.

We developed two fishing grounds (50 m by 50 m) in collaboration with Mie prefectural government in October 2014 (Fig. 8). For one ground we used river sand and for the other ground gravel stones. The median grain size was 0.75 mm for the river sand and 4 mm for the gravel stones. We are monitoring the efficiency of the grounds in terms of growth and survival of asari. About seven months has passed after the release, and so far the growth and survival of asari are good.

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Bull. Jap. Fish. Res. Edu. Agen. No. 42, 75－77, 2016

Abstracts of Poster Presentations

List of Presentations

1: Marine microalga *Rhodomonas* sp. as a novel live food for bivalves (S. Yamamoto, M. Okauchi and T. Yoshimatsu)

2: Observation and monitoring of asari clam predators in a population-collapsed habitat using Field Server (N. Tezuka, N. Kajihara, T. Fukatsu, K. Oguri and T. Yamakita)

3: Seasonal variations of carbon and nitrogen stable isotope ratios in Manila clam, *Ruditapes philippinarum*, in Wajiro intertidal sand flat, Japan (M. Kodama, M. Yamada, S. Yamazaki and N. Ikeda)

4: Predator-prey relationship among 3 species (Manila clam, kuruma prawn, tiger puffer) for stock enhancement program at tidal flat in Japan (A. Yoshida, H. Yamane, H. Yamazaki, A. Itou, K. Sakiyama and Y. Sakakura)

5: Correlation between water nutrient level and catch of asari, *Ruditapes philippinarum*, in fishing grounds in Japan (M. Uchida, M. Tsujino, N. Tezuka, T. Shigeta, Y. Takada, K. Niwa and Y. Ishihi)

6: Long-term fluctuations in stock abundance of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan (K. Hanyu)

7: Effects of small clam abundance, rainfall and sediment on recruitment success of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan (K. Hanyu)

8: Sampling design for abundance estimation of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan (K. Hanyu, H. Kokubu, N. Hata, T. Mizuno, T. Inoue, T. Kudo, N. Hasegawa, Y. Ishihi, S. Watanabe, Y. Fujioka, J. Higano, R. Nambu and H. Kuwahara)

9: Discovery of a spawning ground of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan (K. Hanyu, H. Kokubu, N. Hata, T. Mizuno, T. Inoue, Y. Tanaka, T. Kudo, N. Hasegawa, Y. Ishihi, S. Watanabe, Y. Fujioka, J. Higano, R. Nambu and H. Kuwahara)

10: Ontogenetic changes in ingestion and digestion abilities against diatom cells of the Manila clam (S. Houki, T. Kawamura, N. Ogawa, N.I. Won and Y. Watanabe)

11: Reproductive biology of Manila clam *Ruditapes philippinarum* in the coastal waters along eastern Hokkaido, Japan (Y. Konda and M. Sasaki)

12: Food environment for bivalves around *Zostera marina* beds (H. Kokubu, H. Hanyu and N. Hata)

13: Manila clam, *Ruditapes philippinarum*, culture using *Pyropia* spheroplast in closed recirculation system (N. Arisman, M. N. D. Khan, A. M. Shahabuddin, T. Yoshimatsu, T. Araki)

14: Comparison of types of cages and substrates in suspended culture of the Manila clam, *Ruditapes philippinarum* (N. Hata, N. Hasegawa, T. Mizuno, Y. Fujioka, Y. Ishihi, S. Watanabe, D. Asao, M. Yamaguchi, H. Imai, K. Morita and J. Higano)

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Abstracts of Poster Presentations

1: Marine microalga *Rhodomonas* sp. as a novel live food for bivalves

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Recently, many species of bivalves, including the asari clam have become more popular as seafood all-over the world. In spite of the increasing demands of bivalves, the natural resources are becoming scarce year by year. Therefore, stable aquaculture production of bivalves is highly required to substitute the natural resources. Marine microalga *Rhodomonas* sp. is widely distributed in Japan's coastal waters. This species has a high content of highly unsaturated fatty acids (HUFAs) that enhance the survival and growth of bivalves. For this reason, *Rhodomonas* sp. is expected to play an important role as feed for bivalve aquaculture. In this study, suitable conditions for mass culture of *Rhodomonas* sp. in terms of temperature, salinity and light intensity were investigated. A series of rearing experiments was conducted with one of the three factors altered at a time. In temperature, higher cell increase rate was observed between 16 and 24°C, and the maximum cell density (435.8 × 10^4 cells ml^{-1}) was observed at 24°C. In salinity, higher cell increase rate was observed between 14 and 35 psu, and the maximum cell density (373.9 × 10^4 cells ml^{-1}) was observed at 21 psu. In light intensity, higher cell increase rate was observed between 35 and 80 μ mol m^{-2} s^{-1}, and the maximum cell density (360.1 × 10^4 cells ml^{-1}) was observed at 80 μ mol m^{-2} s^{-1}.

2: Observation and monitoring of asari clam predators in a population-collapsed habitat using Field Server

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The Nakatsu tidal flat, located in western Seto Inland Sea, Japan, is known as an asari clam, *Ruditapes philippinarum*, population-collapsed habitat. A recent study observed low clam survival rates, < 15%/yr, from post-settlement until fishable size in this flat. Based on previous netting/caging experiments, predation has been suggested as a major cause of clam mortality. However, we do not have enough information on predator species composition or when and how often the predators appear on the flat. We employed the Field Server, originally developed for agricultural field monitoring equipped with a web-camera and sensors, to observe the asari clam predators on the Nakatsu tidal flat. Field Server observations, comprised web-camera images retrieved every 30 min via the internet, revealed that ducks (*Anas acuta*) often appear on the monitoring site during low tide in winter. These results suggest that predation by ducks may be a significant cause of asari clam mortality during winter in the Nakatsu tidal flat.

This study was supported by the Ministry of Agriculture, Forestry and Fisheries of Japan.

3: Seasonal variations of carbon and nitrogen stable isotope ratios in Manila clam, *Ruditapes philippinarum*, in Wajiro intertidal sand flat, Japan

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Seasonal variations of carbon and nitrogen stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, in soft body tissue of the Manila clam, *Ruditapes philippinarum*, and their potential food sources were investigated in the Wajiro intertidal sand flat, Fukuoka, Japan. The $\delta^{13}C$ and $\delta^{15}N$ of *R. philippinarum* varied between -15.0‰ and -19.1‰ and between 10.2‰ and 14.0‰, respectively, and they showed a seasonal cycle that was higher from summer to autumn and lower from winter to spring. Carbon to nitrogen (C/N) ratio of the soft body tissue, which is related to glycogen content, also showed a seasonal cycle which became higher in spring and lower in winter, and C/N ratio exhibited a significant negative correlation with $\delta^{13}C$. Comparison of the yearly mean stable isotopic signatures of *R. philippinarum* and those of potential food sources indicates that *R. philippinarum* utilize particulate organic matter in the water column and benthic micro algae as main food sources. These results suggest that the stable isotopic signatures of *R. philippinarum* reflect not only the isotopic signature of assimilated food but also the fluctuation of their body condition related to seasonal storage cycle of glycogen.

4: Predator-prey relationship among three species (Manila clam, kuruma prawn, tiger puffer) for stock enhancement program at tidal flat in Japan

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Hatchery-reared Manila clam (*Ruditapes philippinarum*), kuruma prawn (*Marsupenaeus japonicus*) and tiger puffer juveniles (*Takifugu rubripes*) are released into the same tidal flat, however, they can be in predator-prey relationship at the nursery area. If this is the case, we need to reconsider a releasing strategy. However, it is unclear whether predator-prey relationship occurs among these seedlings. We examined Exp.1) size-related predator-prey relationship between kuruma prawn and Manila clam, Exp.2) size-related predator-prey relationship between tiger puffer and kuruma prawn and Exp.3) predator-prey relationships among 3 species. Exp.1) One kuruma prawn and 5 - 10 Manila clams were placed into a container. We identified shell-length of Manila clam ingested by kuruma prawn after collecting Manila clam survivors after 24 hours. Kuruma prawn (23.1 - 141.2 mm BL) preyed on Manila clam (1.6 - 10.4 mm shell length). Size ratio between prey and predator ranged 1.3 - 9.5%. The size of second maxilliped of kuruma prawn coincided with the maximum size of ingested clam. Exp.2) One tiger puffer and 10 kuruma prawns were placed into a tank (43 cm diameter, 30 cm water depth). After 24 hours, we identified BL of kuruma prawn ingested by tiger puffer after collecting survivors of kuruma prawn. Tiger puffer juvenile (25.7 - 100.3 mm SL) preyed on kuruma prawn (18.6 - 52.2 mm BL). Size ratio between prey and predator was 20.7 - 118.4%. Tiger puffer preyed on kuruma prawn 25 individuals at maximum in 24 hours. Exp.3) Tiger puffer, kuruma prawn and 2 size classes of Manila clam (9.0 cm and 3.2 cm shell length) were placed into a tank (167 × 75 cm, 30 cm water depth) and we counted survival individuals twice a day for 5 days. Next, we set 5 partition nets (4 × 4 m) in a mesocosm, and 3 species were transferred into 3 compartments, and 3

species and black seabream (*Acanthopagrus schlegeli*) were transferred into 2 other compartments. We counted survived individuals 5 days later. Kuruma prawn and Manila clam survived at 43% and 69.5% in the experimental tanks, respectively. In the mesocosm, Manila clam (3.2 mm shell length) greatly decreased in the 3 species mixed experiment. On the other hand, kuruma prawn greatly decreased and Manila clam (3.2 mm shell length) survived when 3 species and black seabream coexisted.

5: Correlation between water nutrient level and catch of asari, *Ruditapes philippinarum*, in fishing grounds in Japan

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Catastrophic decrease of catch of the asari clam, *Ruditapes philippinarum*, has been observed for the last three decades in Japan's coastal waters. Many factors including overfishing, disease, habitat loss, competition with invasive species, global warming, and altered trophic cascade are suggested to be involved. However, major factor is still not well characterized to explain the widespread and long-term decrease of the catch. We conducted comprehensive comparison of environmental and biological characteristics among 17 asari fishing grounds. Significant relationship was observed between water nutrient level (i.e., total nitrogen and chlorophyll *a*) and the asari catch. Significant relationship was also observed between the benthos biomass (macrobenthos, meiobentos, and nematodes) and the asari catch. Stable carbon isotope ratio of asari was found to be a useful indicator representing not only the primary production but also the asari catch per unit area.

6: Long-term fluctuations in stock abundance of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan

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Commercial catch of the asari clam (*Ruditapes philippinarum*) decreased markedly in Japan after the 1980's, and catch in Mie prefecture decreased after the 1990's. In addition, catch in Ise region, southern part of Ise Bay, Mie prefecture, decreased after the 1990's. Mie Prefecture Fisheries Research Institute conducted a survey on the abundance of *R. philippinarum* in a no-fishing area in the southern part of Ise Bay from 1954 to 2000 by direct estimation. The present study identified cohorts of *R. philippinarum* and analyzed long-term fluctuations in the abundance of clams in two size classes (small, shell length of 2 – 7 mm and large, shell length of ≥20 mm) in each cohort in the no-fishing area from 1957 to 2000 based on the data collected by Mie Prefecture Fisheries Research Institute. Forty cohorts were identified in the no-fishing area during the period. The maximum density of small clam in cohorts in the 1950's, 1960's, 1970's, 1980's, 1990's, and 2000's were 6408, 1120, 644, 514, 332 and 331 individuals/m^2, respectively, showing a gradually decreasing decadal trend. The maximum density of large clam in the 1950's, 1960's, 1970's, 1980's, 1990's, and 2000's were 2.8, 5.0, 6.5, 2.7, 3.3 and 2.0 kg/m^2, respectively. The density of large clam decreased markedly after the late 1970's. Therefore, the decreasing trend in the no-fishing area did not synchronize with those in Ise region and Mie prefecture (i.e., 1990's). These results indicate that fishery catch data may not correctly predict actual

fluctuation pattern of stock abundance.

7: Effects of small clam abundance, rainfall and sediment on recruitment success of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan

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The abundance of large (shell length of ≥ 20 mm) asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan decreased markedly after the 1970's. The present study examined effects of small clam (shell length of 2 – 7 mm) abundance, rainfall pattern and sediment on the recruitment success of the cohorts in the no-fishing area from 1957 to 2000 by using logistic regression analysis. The recruitment success was defined as large clam abundance being higher than the median of the entire set of data (355 individuals/m^2). Small clam abundance and rainfall significantly influenced the recruitment success of the cohort, whereas the effect of sediment (i.e. ratio of very fine silt, silt and clay to total weight) was not significant. The logistic regression analysis revealed that the recruitment success was most strongly affected by the maximum monthly rainfall within three months of the peak occurrence of the small clams. The heavy rainfall and consequent river flood seemed to decrease the recruitment success of the clam to the no-fishing area. However, heavy rainfall and river flood (e.g. maximum monthly precipitation of over 500 mm) were often observed not only after the 1970's but also before the 1970's, and the maximum small clam abundance showed a decreasing decadal trend from the 1960's to 2000's, so the decline in stock abundance after the 1970's was considered to be primarily caused by a decrease in abundance of small clams after the 1960's. The very high abundance of small clams in Shitomo River in the western part of Ise Bay during the 1990's was determined to be a result of abundant larval supply (Miyawaki and Sekiguchi 1999, 2000). Therefore, larval supply from spawning grounds to the western and southern part of Ise Bay may have decreased since the 1960's, and this may have contributed to the decline in stock in the no-fishing area and the entire Ise Bay after the 1970's.

8: Sampling design for abundance estimation of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan

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To elucidate the formation process and to develop maintenance techniques of populations of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan, large-scale surveys were undertaken to estimate the abundance of large and small clams in many local populations in the bay. In order to design an adequate sampling technique for estimating the clam abundance on a large scale, we conducted surveys in 7 fishing grounds in Ise Bay and analyzed the data by using a two-stage sampling technique, which is a common method used for estimating the mean, standard deviation and standard error of fishery and ecological data. In the present study, the standard deviations positively correlated with the means both in primary units (sampling points randomly selected in a given fishing ground) and in secondary units (replicates of each sampling point). In addition, the standard deviations in the primary units were larger than those in the secondary units. If we diminish

the ratios of the standard error to the mean (i.e., coefficient of variation of the abundance, which is one of the precision index), a sample size of 2 in the secondary units may be sufficient and an as-large-as-possible sample size in the primary units may be adequate. In practice, in the present surveys with a sample size of > 0.18 points per hectare in the primary units and a sample size of 2 replicates per point in secondary units, the coefficient of variations were not small (i.e., 0.2 – 0.8), but we were able to discriminate the fishing grounds and seasons with large abundance from those with small abundance.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

9: Discovery of a spawning ground of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan

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Abundance of small (shell length of 2 – 7 mm) asari clam (*Ruditapes philippinarum*) in the southern part of Ise Bay, Japan, explosively increases once in several years. However, the location of the spawning grounds, as well as the reason for the large fluctuation in abundance is yet to be discovered. In order to locate the spawning grounds and elucidate the mechanisms of the fluctuations, we conducted surveys of the clam abundance in several regions of Ise Bay, Japan from November 2012 to November 2014. We discovered a high abundance of large clams (shell length of ≥ 15 mm) at a water depth of 5 – 10 m in a non-fishing ground, offshore of Suzuka region, northern part of Ise Bay in November 2013. We subsequently observed a high abundance of small clams inshore of Matsusaka region, southern part of Ise Bay in May 2014. Therefore, this ground may be a spawning ground for the populations in the southern part. The occurrence of such ground with high abundance in the northern part was negatively correlated with the occurrence of inflow of hypoxic water masses during the survey period. The inflow of hypoxic water masses to the ground was often observed by Mie Prefecture Fisheries Research Institute from 2002 to 2009, during which the occurrence of inflow negatively correlated with commercial catch of the clam (shell length of ≥ 32 mm) in the southern part two years later. This supports the hypothesis that the ground discovered in the northern region may be a spawning ground for the populations in the southern part, and the occurrence pattern of hypoxic water masses in the northern region may help understand the population dynamics of the clam in the southern part of Ise Bay.

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10: Ontogenetic changes in ingestion and digestion abilities against diatom cells of the Manila clam

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Planktonic diatoms have long been considered as one of the main food sources for the Manila clam. Whereas, recent studies using stable isotope

analyses suggested that benthic diatoms, which often grow at high densities on the tidal flat and are suspended by waves or tidal currents into the water column, were mainly assimilated by the clams. To assimilate the diatom cell contents, clams need to physically break the silica cell walls of diatoms. In the preceding studies on the feeding of abalone species, the physical strength of the cell walls is an important factor determining the dietary value of diatoms for juvenile abalone. Diatom cells cannot be efficiently broken in the abalone gut, and only highly breakable diatoms when they are eaten, such as those with structurally weak cell walls, have high dietary values for abalone. For the Manila clam, detailed mechanisms in the ingestion and digestion of diatoms have never been investigated, and whether the clam can break the diatom cells in the gut is still totally unknown. In the present study, ontogenetic changes in the ingestion and digestion abilities against diatoms of the Manila clam were examined using several diatom species with different hardness and size of cells. Two planktonic and three benthic diatom species were isolated and cultured from a tidal flat in Tokyo Bay, and the physical strength of their cell walls was measured using the PicoIndenter® (Hysitron). Each diatom species was fed to newly settled (200 – 300 μm) and juvenile clams of 3, 5 and 10 mm shell length (SL) respectively, and the cell walls of diatoms in the tissue section of the digestive tract and feces of the clams were observed using a SEM or TEM. To evaluate the dietary value of each diatom species for newly settled juvenile clams, growth rates of juveniles were measured for a month. The percentage of crushed diatom cells in the ingested cells was measured. The physical strength of cell walls was significantly different among the diatom species, for example, that of the pelagic *Chaetoceros* species was low, and that of the benthic *Navicula* species was high. The newly settled juvenile clams did not crush the benthic diatoms with hard cell walls, and did not ingest large diatoms. Consequently, small pelagic diatoms with fragile cell walls were considered to be suitable foods for the newly settled juveniles. The clams of 3 – 5 mm SL partly crushed the benthic diatoms with hard cell walls. Clams of 10 mm SL ingested large diatoms, and crushed over 90% of ingested diatom cells regardless of the physical strength of the cells.

11: Reproductive biology of Manila clam *Ruditapes philippinarum* in the coastal waters along eastern Hokkaido, Japan

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In eastern Hokkaido, there is a potential for a further increase in production of Manila clam, *Ruditapes philippinarum*, but there is insufficient information about the reproductive biology of the clam necessary for appropriate resource management. Therefore the reproductive biology was studied from June to September 2011 in the coastal waters off Takase, Nemuro in eastern Hokkaido. The condition factor increased during June, and the population maturity rate increased from late June to mid-July. Both variables maintained high value until late August to early September and decreased thereafter. Gonads in active stage and/or partially spawned stage of both females and males were histologically observed in late June, and gonads in these stages were continuously observed during the spawning peak from late August to early September. These results indicate that the spawning of the Manila clam starts before late June and peaks in late August in eastern Hokkaido. Shell length at which 50% of individuals mature was estimated to be 23.6 mm by visual observation of the molluscous part, and to be 20.2 mm for females and 16.3 mm for males by histological observation. Female fecundity was estimated by counting the number of eggs under the microscope, and it was positively correlated to the shell length. The fecundity was also positively correlated to the condition factor. The maximum fecundity estimated in this study was 10^7 eggs for a female with the shell length of 50.9

mm. The current findings will be used to implement resource management proposal in eastern Hokkaido.

12: Food environment for bivalves around *Zostera marina* beds

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Zostera marina is a common seagrass that can be observed in Ise Bay, Japan. *Zostera* beds have important functions as spawning and nursery grounds for valuable marine resources. However, the area of *Zostera* beds in bay area drastically decreased from about 10,000 ha in 1955 to 100 ha in 2000 (one hundredth of 1945) by reclamation for industrial ground and devastation by fisheries trolling device. At the same time the catch of fisheries in Ise Bay has been decreased by about half for 50 years. For this reason, restorations of *Zostera* beds have recently been conducted in coastal environment in Ise Bay. However, few studies have focused on the function of fish-gathering around *Zostera* beds. In this study, we studied the food environment for bivalves around *Zostera* beds at Gotenba and Matsunase beach in Ise Bay. Particulate matter suspended in the water of *Zostera* beds was collected in a sediment trap, and the amount of bivalves was measured in both beaches every season in 2013. Around the margin of the *Zostera* beds, high amount of bivalves were found, and they decreased with the increasing distance from *Zostera* beds. The chlorophyll-*a* level in the particulate matter was the highest at the center of *Zostera* beds and also decreased with the increasing distance from *Zostera* beds. The results suggest that the deposition of particulate matter was increased by buffering of waves and currents by *Zostera* beds. Thus, the food environment for bivalves was improved toward the *Zostera* beds, resulting in increased amount and condition factor of bivalves around the *Zostera* beds.

13: Manila clam, *Ruditapes philippinarum*, culture using *Pyropia* spheroplast in closed recirculation system

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The Manila clam, *Ruditapes philippinarum*, were reared in an indoor closed recirculation system, using *Pyropia* spheroplast feed products, freeze dried spheroplast (FDS), spray dried spheroplast (SDS), and enzyme treated nori (ETN). The study aimed to investigate the compatibility of the recirculation system in order to sustain the Manila clam culture using *Pyropia* spheroplast. Water quality parameters and biological performances were evaluated within thirty days. Experimental results showed that there were no statistical differences among all treatments in water quality parameters of ammonia, nitrite, nitrate and phosphorus concentrations. Water quality on each dietary treatment was under the ambient water quality criteria for the Manila clam. On the biological performance, there were no significant different on survival, feed consumption and growth among the Manila clam feed FDS, SDS and ETN. This result shows that the recirculation system is adequate in sustaining the Manila clam culture in indoor tank.

14: Comparison of types of cages and substrates in suspended culture of the Manila clam, *Ruditapes philippinarum*

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A series of experiments was conducted to determine optimal rearing conditions for suspended culture of the Manila clam, *Ruditapes philippinarum*, in Ounoura Bay, Mie prefecture, Japan. Comparisons of four types of cages (plastic container, cylindrical net cage, small mesh cage, curing cage used for pearl oyster) and two types of substrates (gravel and pumice) showed that the combination of plastic container with gravel and the combination of cylindrical net cage with pumice had the fastest growth rate of the clam. In terms of handling, however, cylindrical net cage with pumice was considered superior due to its lighter weight. A rearing experiment with different amount of the clam and pumice in the cylindrical net cage (45 cm diameter) showed that installing two mesh bags each containing 0.5 kg of the clam and 5 L of pumice had the best growth rate. The growth of the clam was faster in inner part of the bay than in the bay mouth at the depth of 4 m among the three culturing depths (1 m, 2 m and 4 m), and chlorophyll *a* level seemed to be one of the factors affecting the growth rate. The condition factor (CF, soft tissue weight / shell volume) of the clam cultured in cylindrical net cages ranged from 18.0 to 22.9 from December to March, which increased from 16.5 at the initiation of the experiment in October.

15: Effects of feeding regimes on oocyte quality of Manila clam *Ruditapes philippinarum*

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In the seed production of Manila clam, *Ruditapes philippinarum*, low survival and retarded growth of the larvae sometimes occur. One of the possible causes for these troubles is the inferior quality of mature oocytes (eggs) spawned to be fertilized. Insufficient feeding of the female brood stocks is expected to deteriorate the oocyte quality, but experimental studies to clarify the effects of feeding on the oocyte quality are scarce. In this study, we examined effects of three different feeding regimes on the quality of mature oocytes in Manila clam. Two hundred Manila clams (mean shell length 30 ± 1.7 mm) in the spawning season (October) were divided into three groups (group H, M and L) and reared in tanks at 22°C for 37 days. They were continuously fed with *Chaetoceros neogracile* at 6.0 × 10^4 cells/ml for the first 7 days. On day 8, the feed concentration was changed into three different levels (H, 8.0; M, 1.6; L, 0.3 × 10^4 cells/ml) and the rearing was continued for the rest of the experiment period. On days 28 and 37, a part of the clams from three groups were induced to spawn by an exposure to increasing water temperature. Females and males that started gamete release were immediately transferred to a small container individually. The eggs collected from each female were inseminated with the pooled sperm obtained from three males, and the total number of eggs from each female was counted. Then a part of the fertilized eggs from each female was transferred to 3 wells of a 24-well microplate at 60–100 eggs/well and to 24 wells of a 48-well microplate at 3–4 eggs/well, and kept at 25°C. At 4–6 hours after the insemination, eggs in the 24-well plate were fixed with 5% formalin/seawater to obtain the fertilization rate. At 22–23 hours after the insemination, larvae in the 48-well plate were fixed in the same way, and the number of veliger larvae was counted to obtain the metamorphosis rate. In addition, hinge length of the fixed larvae was measured. Average feed concentration in the waste water (H, 1.7; M, 0.5; L, 0.17 × 10^4 cells/ml) confirmed difference in feed availability among the experimental groups. The difference in the feeding regimes, however, did not show significant effects on the number of the spawned eggs, the fertilization rate, the metamorphosis rate, or the hinge length of the veliger larvae. These results indicate that limited

feeding (i.e. group L in this study) for about one month during spawning season does not deteriorate the oocyte quality in Manila clam. Large variation of the observed parameters within each group suggests presence of other factors that affect the oocyte quality.

16: Low-salinity endurances of the penshell *Atrina* sp. and clam *Trapezium liratum*

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Species-specific and temperature-dependent endurances against low salinity were revealed for the bivalves *Atrina* sp. and *Trapezium liratum* in a 4-day consecutive laboratory experiment with an orthogonal design: 12 levels of salinity ranging from 0 to 100 psu × 2 bivalve species × 2 temperatures, 12 and 24°C. [Result 1] *Atrina* sp. was highly vulnerable to low salinities compared to *T. liratum* (mean survival rate in salinities ≤ 20 psu: 11% and 94%, respectively). This conforms to the interspecific difference such that, of the two species, *Atrina* sp. inhabits deeper water where abrupt decrease in salinity is relatively rare. [Result 2] Both species showed decreased survival rate and/or survival time at a higher temperature. This accords with the results of additional experiments that both species increased clearance rate at higher temperatures owing presumably to increased respiration and were likely to expose their fragile soft tissue to more low-salinity water.

17: Effect of netting on survival of Manila clam *Ruditapes philippinarum* in an artificial tideland in Okayama, Japan

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In Yorishima artificial tideland located in Seto Inland Sea, small Manila clams (*Ruditapes philippinarum)* tend to be found in spring but disappear by autumn. Therefore, these clams might suffer high mortality during summer. In this study, the effect of netting of their habitat on survival of Manila clam was examined in Yorishima artificial tideland. Prior to netting treatments, spatial distribution of Manila clam in Yorishima artificial tideland was surveyed with line transect sampling. A core sampler (10 cm in diameter and 8 cm in depth) was used to measure the density of clams. A total of 160 plots were surveyed and clam density ranged from 0 to 4318 clams/m^2. Five plots with high clam densities (2159 to 4318 clams /m^2) were assigned as netting treatment plots and covered with nets (2.5 × 2.5 m, 4 mm mesh openings) in May 2014. Net edges were buried 20 cm deep. A no-net control plot was placed adjacently to each netting treatment plot. In October 2014, the clam densities in three netting treatment plots were higher than those in no-net control plots. These results suggested that the netting treatment was effective to protect the clams from waves and/or predators in Yorishima artificial tideland.

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18: Effects of water temperature on the expression of heat shock protein (HSP) 22, 40, and 70 genes in Manila clam (*Ruditapes philippinarum*) larvae

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Biomarkers are powerful tools to understand optimal environmental conditions for the rearing of target animals. The heat shock protein (HSP) family is one of the biomarkers, which responds to various stress factors. We examined *HSP 22-1*, *22-2*, *40*, and *70* expressions in the Manila clam (*Ruditapes philippinarum*) larvae in different thermal conditions. In experiment (Exp.) 1 (short-term stimulation), the fertilized eggs were collected by thermal spawning induction and were hatched at 23°C. D-shaped larvae [1 day after fertilization (DAF)] were transferred into 3 tanks at 20–22, 25, or 30° C, and were sampled after 24 h. In Exp. 2 (long-term stimulation), D-shaped larvae were reared in the same thermal conditions as described for Exp. 1, and sampled at the ambo (5–7 DAF) and full-grown stages (9–12 DAF). All samples were soaked in RNA preservative solution and stored at −80°C. Expressions of *HSP*s were quantified by real-time PCR. In Exp. 1, *HSP 22-1* and *22-2* expressions significantly increased at 25 and 30°C. *HSP 40* expression significantly decreased at 30°C, and *HSP 70* expression showed no significant changes. In Exp. 2, *HSP 22-1* expression in full-grown larvae at 25 and 30°C, and *HSP 22-2* expression in the ambo and full-grown larvae at 25 and 30°C increased significantly. *HSP 40* expression in ambo and full-grown larvae in all temperature regimes decreased significantly, and *HSP 70* expression in ambo larvae in all temperature regimes increased significantly. These results suggest that *HSP 22-1* and *22-2* are suitable biomarkers of thermal stress in Manila clam larvae.

19: Spatiotemporal population structure of the asari clam *Ruditapes philippinarum* juvenile in Ise Bay inferred by microsatellite marker analysis

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The production of asari clam *Ruditapes philippinarum* fishery has decreased severely in Ise Bay. To distinguish the spatiotemporal population structure of the clam for the better resource management, we sampled juvenile clams (shell length < 10 mm) in May and November 2013 and May 2014 at 6 to 7 sites in Ise Bay. For microsatellite analysis, total DNA was extracted from 23 to 32 individuals from each sampling location at each sampling period. The microsatellite analysis was performed on six loci (Asari 16, Asari 43, Asari 62, Asari 64, KTp8 and KTp31-1) to reveal the genetical population structure of the clam in Ise Bay. Calculations of molecular variance and

pairwise F_{ST} test were done using the software ARLEQUIN. No significant population subdivision was detected between the May 2013 and May 2014 specimens in all of 49 pairs by pairwise F_{ST} test. However, significant pairwise F_{ST} differences were observed in 25 of 42 (0.015 – 0.048, $p < 0.01$) pairs between May 2013 and November 2013 and 26 of 42 (0.015 – 0.055, $p < 0.01$) pairs between November 2013 and May 2014. Assignment analyses using the software STRUCTURE identified $K = 1$ as the most probable number of population. Genetic relationship among the samples collected from different locations at each sampling period was also estimated with the software PHYLIP using the neighbor-joining method. The samples were divided into 2 clusters comprising 4 groups from November 2013 specimens and the other 17 groups while the Bootstrap value was 56. These results suggested the main sources of the juvenile clams may differ between May and November in Ise Bay.

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20: Present situation of the asari clam *Ruditapes philippinarum* resources in Mikawa Bay, Japan

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The annual catch of the asari clam in Aichi prefecture amounted approximately to 16,000 tons, occupying about 70% of the national catch of Japan in 2013. The national catch of Japan has decreased by 80% in 30 years, whereas the catch in Aichi has been maintained above 10,000 tons annually for the last 30 years. We suggest the following factors contribute to the stable asari clam resources in Aichi. (1) Wider tidal flat and shallow areas for the asari clam habitats. Fishing grounds of the asari clam are reported in depths shallower than 10 m close to the flats. (2) Availability of seeds on the tidal flats around river mouths. (3) Transplantation of juvenile clams for stocking from the seed areas into various areas within the bay. (4) Planktonic larvae with high density from spring to early winter. (5) Fishery managements by the local fishery associations, including the transplantation, restriction on the shell size and amount of commercial catches, setting of no-fishing periods and areas, removal of predatory and competitive organisms, tilling of the fishing grounds, and also prohibition of stocking juveniles from the above areas. In particular, factors (1) to (4) are important for the formation, maintenance, and enhancement of the asari clam resources to maintain population network of the asari clam within the bay thorough the life cycle. The asari clam spends most of its lifecycle on tidal flats and the neighboring shallow areas, so we need to keep environmental conditions suitable for the asari clam. Currently, the supply of juvenile clams for the transplantation is dependent upon limited seed areas. We assume that juvenile clam aggregations in the seed areas are generated largely by the characteristic bottom water flow patterns at river mouth areas.

21: Seasonal dynamics of the parasitic larvae and free-living adults of the sea spider *Nymphonella tapetis* infecting the asari *Ruditapes philippinarum* in the eastern coast of Chita Peninsula.

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We investigated infection of the Manila clam

Ruditapes philippinarum with larvae of the sea spider *Nymphonella tapetis* and distribution density of free-living adults of *N. tapetis* on the eastern coast of the Chita Peninsula, Japan. The prevalence of infection and mean abundance of parasitic larvae per host decreased in May or June when many adults were released from the host and peaked in June or July when many young larvae had parasitized *R. philippinarum*. Subsequently, the prevalence of infection and mean abundance of parasitic larvae per host decreased sharply between August and October. After October, only a few larvae of *N. tapetis* invaded *R. philippinarum*. Invasion after October was considered to contribute to the main reproductive cycle that occurred from May onward. The prevalence of infection and mean abundance of parasitic larvae per host fluctuated each year. High survival rate of larvae in summer 2009, which was caused by lower water temperature than in other years, was considered to be the factor of the increase in *N. tapetis* larval numbers from October 2009 to March 2010.

22: Shellfish production and new approaches in Turkey

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Turkey is a large, roughly rectangular peninsular country bridging Europe and Asia. This peninsula is surrounded by the Mediterranean Sea (south part), the Black Sea (north part) and the Aegean Sea (west part). The Sea of Marmara, the Bosphorus and the Dardanelles which are the Turkish Straits demarcate the boundary between Thrace and Anatolia. Turkey has 8333 km of coastline with different ecological properties, so it is one of the best countries having suitable conditions for aquaculture and fisheries. Fishery production consists of marine fisheries (56%), marine and freshwater aquaculture (26%), inland fisheries (11%) and other marine species fisheries (7%) such as crustaceans and molluscs. Aquaculture is a relatively new industry in Turkey. While the total fisheries production has remained stable over the last decade, aquaculture production has been increasing rapidly. Rainbow trout (*Oncorhynchus mykiss*), seabass (*Dicentrarchus labrax*), gilthead seabream, (*Sparus aurata*) and carp (*Cyprinus carpio*) are the main aquacultured fish species. Shellfish production is mainly from capture fisheries except mussel production in a few amounts within a short period. An economical species are *Venus gallina, Ruditapes decussatus, Mytilus galloprovincialis, Ostrea edulis, Venus verrucosa, Donax trunculus,* and *Chlamys glabra*. Moreover *Ruditapes philippinarum* was recorded from Turkish waters in 2003. This species is collected irregularly from Çardak Lagoon-Çanakkale, and the catch statistics is combined with that of *Ruditapes decussatus*. Republic of Turkey Ministry of Agriculture and Rural Affairs arranges some legislations and guidelines for safety and sustainable bivalve production in Turkey. Also regulation and control cover from harvest to process (process techniques), as well as transport and marketing. Totally, 32 regions are monitored regularly for bivalve production, and 8 regions have been investigating in order to start bivalve production in Turkish coastline. There are 12 commercial companies with a depuration plant that export shellfish. Recently, several enterprises have started to culture mussel and also some official permission has been completed for the production especially along the coast of the Marmara Sea. Clam and oyster cultures are expected to be accelerated in Turkey.

23: Variations of glycogen, protein and lipid contents depend on size of *Ruditapes decussatus* in Bostanli Seashore Izmir Bay, Aegean Sea, Turkey

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Biochemical composition of bivalves is important

factor for understanding physiological activities such as feeding, growth and reproduction. Energy is stored prior to gametogenesis in the form of glycogen, lipid and protein. Furthermore, environmental factors particularly food availability play a dominant role in the level of biochemical components. The aim of this study was to compare biochemical contents of the Carpet shell clam, *Ruditapes decussatus*, among different sizes. Carpet shell clam samples were collected monthly between May 2013 and April 2014 from Bostanli seashore, Izmir Bay in Turkey. The samples were divided into three size groups; Group I from 20.0 to 25.0 mm, Group II from 30.0 mm to 33.0 mm and Group III from 45.0 mm to 51.0 mm. At the same time, temperature, salinity, chlorophyll a, total particulate matter were measured each month. Temperature ranged between 11.0°C (in December) and 26.0°C (in June and August) during the study. Salinity was measured between 35.68 ‰ and 37.44 ‰ in the study area. Total particulate matter and chlorophyll a concentration changed from 1.7 mg/l to 8.4 mg/l and from 0.21 µg/l to 19.68 µg/l, respectively. The maximum glycogen value was determined in all groups in September: 18.36% (Group I), 18.86% (Group II) and 20.81% (Group III). Protein and lipid contents in Group I had the highest value in May 71.66% and 8.21%. The maximum lipid level in Group II and Group III was found to be 6.74% and 5.98% in April. The highest protein content in Group II and Group III was 61.67% in April and 63.01% in March, respectively. The results of this study showed that the size and glycogen ratio increased synchronously, whereas protein and lipid contents decreased to relatively larger extents within sampling period. It is thought that this situation is closely related to not only water temperature and food availability but also reproduction and spawning period.

24: Use of *Pyropia* spheroplasts as a live food substitute for culturing Manila clam, *Ruditapes philippinarum*

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An experiment was conducted to assess the *Pyropia* spheroplasts as a live food substitute for culturing Manila clam in indoor flow through system. *Pyropia yezoensis* (red algae) is one of the most important aquaculture species that contains a higher percentage of protein, as well as minerals and vitamins. Spheroplast is a cell from which cell wall is removed by enzymatic treatments. Three different kinds of cell wall degrading enzymes (i.e., agarose, β-1-3 mannan agar and β-1-3 xylanase) were used to breakdown the cell wall. Four diets (PS inclusion levels of 1%, 2% and 3% body weight basis, and live diatom *Chaetoceros calcitrans*) were fed to the three replicate groups of clams for 9 weeks. Significantly higher survival was obtained in the PS diets than the diatom group ($P < 0.05$). Manila clam cultured with PS level 2% diet showed higher carcass weight (soft tissue) compared to the other PS diets and was not different than diatom ($P > 0.05$). Results of the experiment indicated that PS is a good candidate for a substitute to live diatom for culturing Manila clam.

25: Main ecological processes for the determination of *Ruditapes philippinarum* population size on an extensive intertidal sandflat in Ariake Sound, Kyushu, Japan

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For the sustainable use of fishery resources under a proper ecosystem management program, it is necessary to understand the role of main ecological processes in community organization in which the target species population is involved. These include predation, competition, adaptation and limitation

by habitat carrying capacity. In the present study, by combining field monitoring with field and laboratory experiments, we attempted to extract several ecological processes that can primarily determine the population size of the Manila clam, *Ruditapes philippinarum*, on an extensive intertidal sandflat in Ariake Sound, western Kyushu. In 2004, the macrobenthic community on the sandflat was mainly composed of five phytoplankton-feeders, two powerful bioturbating shrimps, *Nihonotrypaea japonica* and *Upogebia major*, and three veneroid clams, *Ruditapes philippinarum*, *Mactra veneriformis* and *Meretrix lusoria*. The results of the field survey suggested that the five species competed for space, with superiorities exerted by shrimps to clams through the former's intense bioturbating activities and by one or two species of clams to the other in specific tide zones, and for food to the limit of the carrying capacity provided by the sound. The two shrimp species populations, which predominated over the high- and mid-tide zone, considerably declined almost to the extirpation during 2004 to 2008. This event was followed by the higher recruitment and biomass increment of the three clam species populations than before, most probably owing to competitive release from the shrimp. Thereafter, only *Me. lusoria* and *Ma. veneriformis* were successful in establishing their adult populations in the newly vacated habitat (i.e., with no shrimps). The sediments there were more unstable than that of the low-tide zone where *R. philippinarum* population persisted. We hypothesized that some high burrowing ability of juvenile clams is required for their persistence in the unstable sediment, especially in the wintertime. The results of the field and laboratory experiments suggested that burrowing ability can be one key trait for success/failure in the population establishment of clam species in the newly vacated habitat. These findings could be used as a basis for the ecosystem-based management of Manila clam populations in well-mixed shallow estuaries.

26: Age determination of asari *Ruditapes philippinarum* using growth rings on shell surface and section in Mutsu Bay, northern Japan

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Asari has scarcely been caught for commercial aim in Mutsu Bay, northern Japan. Hence, productivity of asari in the bay is largely unknown. In the recent summers, Mutsu Bay has sometimes encountered higher water temperatures than observed in the past. To enhance fisheries production in such environmental condition, the species tolerant for the warm water should be efficiently used. As the first step for stock assessment of asari in Mutsu Bay, we explored the age characteristics on its shell surface and section. The specimens were collected between April 2014 and March 2015 from two locales in Mutsu Bay: Ashizaki Bay and Noheji. First, we observed the shell outer surface and found dark-olive colored bands accompanied by notches. Second, the shell was embedded in epoxy resin, and then sectioned into ca. 300 μm thick along its axis of maximum growth. When the thin section was observed under a light microscope, translucent lines started from umbo and passed through middle layer of shell. Each line finally reached a notch on shell outer surface. Thus, the dark-olive colored bands with notches on shell surface were assumed to be "outer growth rings". However, seasonal changes in the structures of the ventral margins of shell sections suggested that the translucent line was mainly formed in August (summer) and also in December (winter). Some translucent lines on shell sections branched off in middle layer of shell and reached nearby the shell outer surface. We used only the ventral one of those as "inner growth rings" to avoid overestimating age. The mean shell lengths (SLs) at the first, second and third growth rings

were estimated to be 10, 22, 30 mm based on the outer growth ring and to be 14, 27, 32 mm based on the inner one, respectively. The observed maximum ages were 7 years old based on the outer growth ring and 5 years old based on the inner one. The results suggest asari reach SL 30 mm (commercial size) in around 3 years in Mutsu Bay, although further evidence should be collected to establish the criterion of outer and inner "growth rings" as age characteristics.

27: Abundance of planktonic larvae of the asari clam *Ruditapes philippinarum* around fishing grounds in Mikawa Bay

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Densities of planktonic larvae of the asari clam were monitored by fluorescent antibody method in Mikawa Bay, which has the largest asari clam resource in Japan, from 1999 to 2008. Monitoring stations were set in 4 areas near Isshiki tidal flat, Fukue Bay, Toyogawa river mouth and Yahagi river mouth; the exact location of the stations differed from year to year. Former 2 sites are the main fishing grounds in Mikawa Bay and latter 2 sites are known as high level juvenile occurring areas. The larvae occurred between spring and autumn every year with varying densities from 10^2 to 10^4 individuals per m^3. The occurrence peaks were found in different time from year to year and area to area. The years and areas with apparently two occurrence peaks in spring and autumn were not common. The D-shaped stage larvae occurred at density of 10^3 individuals per m^3 on the average, whereas full grown stage larvae at 10^2, and thus the survival rate during planktonic stage was estimated to be about 10%. Significant correlation in D-shaped larvae densities was not found among stations except for between Isshiki tidal flat and the neighboring Yahagi river mouth. This fact indicates that the spawning periods of the clam are different among different habitats in Mikawa Bay. It was considered that the high level of juvenile occurrence in Toyogawa river mouth is attributable to the high survival rate after settling rather than high larval supply because abundance of the full grown stage larvae in this area were not particularly higher than in other areas.

28: Population status of Manila clam, *Ruditapes philippinarum*, and physical environmental conditions in the Natori River estuary in northeastern Japan after the Great Tsunami

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The Great East Japan Earthquake and tsunami in March, 2011, resulted in ground subsidence, deposition of rubble and mud. Severe morphological changes occurred in the Natori River estuary in northeastern Japan, damaging bivalve fishing grounds. To clarify the population status of the manila clam, *Ruditapes philippinarum*, and physical environmental conditions, surveys have been proceeding regularly since the tsunami. Population density was estimated based on a quadrat method. Water temperature and salinity were measured in the field whenever sampling was conducted. To obtain accurate diurnal changes in salinity, data-loggers have been installed since 2011. Bottom sediments are also collected and analyzed at regular intervals. Two years after the tsunami, the manila clam population in the Natori River recovered temporarily: many juveniles were collected during the period from autumn, 2012, to the spring of 2013. However, a marked reduction in the population

was recorded after heavy rainfall in the summer of 2013. Manila clam recruitment has not yet occurred successfully since the tsunami. It is suggested that low salinity has induced the damage to the manila clam population. The data logger recorded salinity as low as 0 psu during flood tides. Heavy rain and dam discharge to relieve high reservoir levels have induced longer periods of fresh water detention in this part of the river. Topographical changes, especially the formation of sand spit intrusions into the river, have reduced the width of the river mouth. The destruction of a training wall at the river mouth appears to be the cause of sedimentation. In contrast, the abundance of another clam species, *Nuttallia olivacea*, has returned to approximately the same level as before the tsunami. This bivalve is a euryhaline species, which inhabited the same region in the Natori River estuary as *R. philippinarum* before the tsunami.

29: Dynamics of the sea spider parasitism on asari in Matsukawaura Lagoon, Fukushima, Japan

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The parasitic sea spider *Nymphonella tapetis* is recognized as one of the harmful organisms for Manila clam (asari) *Ruditapes philippinarum* in Japan. In 2007, sudden outbreak of *N. tapatis* occurred and probably caused mass mortality of Manila clam in Tokyo Bay. Then, only three locations in Japan (Tokyo, Aichi, and Fukushima) have been found as the habitat of *N. tapatis* during 2007 - 2011. In Matsukawaura Lagoon, Fukushima, *N. tapatis* was first found in 2009 from wild Manila clam, and thereafter rapidly spread into almost entire lagoon. On 11 March 2011, a huge tsunami caused by the Tohoku-Pacific Earthquake hit the lagoon and greatly altered its environment and landscape. After that incident, the prevalence of *N. tapatis* greatly reduced from 2011 - 2012, and finally *N. tapatis* disappeared from the lagoon in 2013 whereas the Manila clam density has greatly increased. This result implies that *N. tapatis* population may collapse when environmental condition greatly changes despite host clams densely inhabit.

30: Life history and distribution of a sea spider, *Nymphonella tapetis*, parasitic on the Manila clam, *Ruditapes philippinarum*, in Tokyo Bay, Japan

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Nymphonella tapetis is a sea spider that is endoparasitic on bivalves including the commercially important Manila clam *Ruditapes philippinarum*. In 2007, it showed a sudden outbreak in a narrow area of Tokyo Bay and brought serious fishery damage. Because the occurrence of this parasitic sea spider from the shallow coastal areas of Japan had scarcely been reported, knowledge on its fundamental ecological characteristics was limited. Under such circumstances, we first established the monitoring methods for quantitative capture, length measurement and identification of developmental stages. In order to reveal the life history and distribution pattern, monthly monitoring using these methods was conducted on the Manila clams and parasitic sea spiders in Banzu and Futtsu tidal flat of Tokyo Bay from 2010 to 2011. The seasonal occurrence pattern of the parasitic sea spider showed a clear tendency, i.e., higher in summer and lower in other seasons. Based on the monthly changes in the composition of growth stages of the parasite, its primary reproductive season was considered to be in early summer. On the other hand, low recruitment of larvae in hosts was observed almost throughout the year, except for May. We suggest that this year-round continuous recruitment may contribute to a massive increase of the parasite densities in Tokyo Bay and the other regions (e.g., Mikawa Bay).

31: Distribution, morphology and mode of life in the sea spider *Nymphonella* spp. (Arthropoda, Pycnogonida): potential harmful endoparasitism on asari outside Japan

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Sea spiders or pycnogonids are a distinct monophyletic group of exclusively marine arthropods, consisting of about 1,300 described species (ca. 160 species are recorded from Japan's waters). The taxonomic position of sea spiders has been controversial for a long period, but most recent phylogenetic analyses support their class status in the subphylum Chelicerata. Although the information on their mode of life is still insufficient, some species show a close association with soft-bodied invertebrates such as sponges, cnidarians, annelids and molluscs including apparent cases of parasitism. *Nymphonella* is a curious form of sea spider genus composed of the following three (or two) species: *N. tapetis* Ohshima, 1927 recorded from Japan, *N. lambertensis* Stock, 1959 from southern Africa, and *N. lecalvezi* Guille and Soyer, 1967 (often synonymized to *N. tapetis*) from the Mediterranean and Namibia. The adult morphology is very similar among these species, but the parasitism is known only in Japanese *N. tapetis*, which caused serious fishery damage on asari in recent years. In this species, larvae show endoparasitism on bivalves whereas adults live freely on or just under the surface of sandy bottoms. Although the mode of life in other species is not yet fully studied, the sharing of some distinct apomorphies such as the markedly modified second head appendages (palps) and first walking legs raises a possibility of endoparasitism on bivalves, and thus a potential harmful nature of *Nymphonella* species outside Japan. Accordingly, we would like to point out the possibility of prospective fishery damage on asari and other commercial bivalves by means of the endoparasitic *Nymphonella* larvae especially in the eastern Asian and the Mediterranean coastal countries where the aquaculture of bivalves is highly active.

32: Larval and juvenile dynamics of the Manila clam *Ruditapes philippinarum* in northeastern Japan

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Investigations of the larval Manila clam *Ruditapes philippinarum* were conducted weekly or fortnightly in Matsushima Bay from 2012 to 2014 and Mangoku-ura Inlet from 2013 to 2014. Monthly field investigations of the juveniles were conducted at 13 stations in Matsukawa-ura Lagoon from June 2011 to December 2014. Results of larval investigations showed considerable short-term variability of larval densities from June to October. The peak of larval density was observed usually from mid-July to early-August and the second peak occasionally occurred in September. The highest larval density observed in the present study was comparable to that of other major clam habitats in Japan. Therefore, reproductive potential of the clam populations in Matsushima Bay and Mangoku-ura Inlet was considered to be high. Recruitment of juvenile clam (>1 mm SL) was observed in Matsukawa-ura Inlet in December 2011 and from August to February in 2012 and 2013. Early juveniles grew slowly with decreasing their density in winter season and their growth rate rose sharply from spring. Mortality of juvenile clam was observed in summer in 2012 and 2013. Although mortality was not observed in 2014, the growth of juveniles was very slow from August to December. The juveniles which settled in previous year reached almost 20 mm in December 2014. Larval supply and the recruitment of the clam seemed to be basically favorable in the clam habitats in northeastern Japan. However, substantial annual variability in juvenile survival in summer would be critical for the dynamics of the clam populations in northeastern Japan.

33: Condition index and reproduction of the Manila clam *Ruditapes philippinarum* in suspended and bottom culture

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Bivalve suspended culture offers a number of advantages over bottom culture. In this study, we compared the condition index and gametogenesis of the Manila clam *Ruditapes philippinarum* between suspend and bottom culture. We suspended the clam in a plastic container with gravel at a depth of 2 m from a raft in Gokasho Bay, Mie and cultured the clam in a mesh bag containing gravel placed in a tidal flat adjacent to the raft. Samples from each culture were collected monthly from September 2012 to May 2013. The soft tissues were removed from their shells and fixed for histology in Davidson fluid. The tissue samples were embedded in paraffin wax and 5 μm sections were stained with hematoxylin and eosin. The prepared microscope slides were examined to determine sex and stage of reproductive development. Clam reproductive maturity was categorized into six stages: undifferentiated, early developing, late developing, ripe, spawning, and spent. At the start of the study, in September 2012 when clams reached shell length of 15.9 mm (mean, n = 15), most individuals were in a gonadal resting period. There was an increase of the condition index from February to May 2013 in suspended culture, while the index of clams on bottom culture reached the highest value in March and decreased in April and May 2013. Spawning on bottom culture appears to have occurred by May 2013 as suggested by the presence of spent individuals in the May sample. However, spawning in suspended culture appeared to continue from March to May 2013. By May 2013, the majority of samples was in the ripe and spawning stages. It appears from these observations that the spawning period was extended in suspended culture.

34: Report of a novel flagellated parasite in the Manila clam *Ruditapes philippinarum* on the west coast of Korea

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The Manila clam *Ruditapes philippinarum* is endemic to the Yellow Sea of Korea and China and commercially important in this region. Mass mortality of the Manila clam has occurred since mid 1990s, and the protozoan parasite *Perkinsus olseni* has been regarded as one of the causative agents for the mortality due to its rapid infection and severe pathogenicity. In the present study, I report an unidentified protozoan parasite (MPX) in the Manila clam collected from west coast of Korea. Because MPX was morphologically similar to *P. olseni* and co-parasitizing Manila clam with *P. olseni*, MPX was isolated and diagnostic methods for *P. olseni* were applied to distinguish MPX from *P. olseni*. When trophozoite of MPX were incubated in Ray's fluid thioglycollate medium (RFTM) for 2 weeks and staining with Lugol's iodine, cell enlargement and dark brown spheres were observed as like *P. olseni* but lysed with 2M NaOH, suggesting that cell-wall composition of MPX is different from *P. olseni*. The zoospore of MPX observed by SEM averaged 2.38 μm in body length with 6.03 μm of anterior flagellum and 4.37 μm of posterior flagellum. Mastigonemes were found on the anterior flagellum with bilateral array. In PCR diagnosis, MPX was found to spread in the Manila clams collected from the south and west coasts of Korea but not found in the clams from east coast. Clams injected with MPX trophozoites showed 50% of mortality within a week, suggesting that MPX has high pathogenecity in the Manila clam.

35: Geographical distribution of mitochondrial COII haplotypes in the brackish water clam, *Corbicula japonica* (Yamato-shijimi), around the Japanese archipelago

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Genetic structure of the brackish water clam, *Corbicula japonica*, around the Japanese archipelago and adjacent areas were investigated using partial mitochondrial COII (990 bp) gene sequences. 142 haplotypes were detected among 283 individuals collected from 52 sites (260 individuals) in Japan, one site (eight individuals) in Sakhalin Island and three sites (15 individuals) in South Korea. Both of neighbor-joining and maximum likelihood phylogenetic trees revealed the presence of two major groups (Group I and II). Group I was monophyletic, and comprising five monophyletic lineages (Lineages A-E) of *C. japonica*, while Group II including *Corbicula* sp. was paraphyletic. Mean pairwise genetic distance between Group I and II was 1.9%, and they were conspicuously distinct in shell coloration. Lineages A-C were recognized in Japan and Sakhalin Island, while Lineages D, E and *Corbicula* sp. were found in the Korean peninsula. Lineage A was dominant in Japan. Nested clade analysis (NCA) revealed three sublineages in Lineage A, which were distributed in Pacific Ocean, northern and southern regions of Japan Sea, respectively. In sublineages, ancestral haplotype was commonly observed throughout their entire distribution, with 110 haplotypes unique to single localities in Japan and Sakhalin Island. Mismatch distribution of Lineage A showed the population expansion in the near past. Lineage B was restricted to a single locality on the western coast of Kyushu. Lineage C was mainly observed at the areas between Sendai and Tokyo Bays. Lineages D and E were distributed in the eastern and southern coasts of the Korean

peninsula, respectively. Geographical distribution of these lineages appears to have been influenced by major ocean currents around the Japanese archipelago. The ubiquity of ancestral haplotypes and the pattern of mismatch distribution indicate that early stages of range formation of *C. japonica* were characterized by rapid dispersal influenced by ocean currents. In contrast, the presence of many haplotypes specific to single locality suggests that current gene flow among extant populations is rather limited.

36: Effect of different artificial sea waters on survival and growth of adult Manila clam, *Ruditapes philippinarum*

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We investigated the influence of water-types (T_1: Sea water, T_2: Distilled water with 3% NaCl, T_3: Deionized water with 3% NaCl, T_4: Deionized water containing 3% natural sea salt and T_5: Tap water mixed with 3% NaCl) on survival of adult Manila clam, *R. philippinarum* in a twenty-day experiment. The adult clams were reared in plastic mesh cages at 6 individuals per cage with three replicates. One diet (PS: *Pyropia* spheroplasts) was fed to the test clams two times a day (10:00 and 15:00) at the rate of 0.25% body weight per day in a closed water system. Survival and growth of shell size were taken into consideration for evaluating dietary performance. A significantly ($P < 0.05$) higher survival was obtained in T_1 followed by T_4, T_5, T_3 and T_2, indicating the superiority of natural sea water over other forms of water in spite of having the same basic nutritional value and exhibiting the deficiencies of certain salts considered to be indispensable for clams` life and survival.

37: Method for detection of colored sand using image analysis and its application to monitoring sediment movement on a tidal flat

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Field investigation of sediment movement in estuarine area is necessary to elucidate not only the sediment dynamics and tidal-flat geomorphology but relation between estuarine morphology and ecological environment. In coastal areas, there are sand transport studies and observations by use of colored and/or fluorescent sand as a tracer. For detecting the colored tracer from sediment samples, human visual inspection has been conventionally used, thereby a huge cost is required to the tracer survey. Frequent and multipoint tracer surveys to understand coastal and estuarine morphodynamics need a low-cost method for detection having satisfactory the accuracy. In this study, we developed a method of colored sand detection using computer-assisted digital image analysis technique and human visual inspection. These digital images of sediment samples were taken by flatbed scanner from papers which were pasted the sand. The colored sand detection was carried out by image analysis. In the final stage of this procedure, check of trimmed-image outputs as the results of image analysis by human visual inspection was employed to eliminate the erroneous detection and to increase its accuracy. This method was time-saving in the tracer detection from a large number of the samples, therefore it was suitable for the multipoint tracer survey. In addition, we applied this method to field observation in a tidal flat of the Toyo River estuarine (Rokujo-gata), Aichi, Japan. This observation was carried out for 9 months, from September 14, 2012 to June 18, 2013 to grasp characteristics of sediment movement in the tidal flat. The sediment samples were collected at 18 points within a spatial coverage extended 1 km alongshore and 800 m cross-shore direction on the tidal flat and once a month during

the observation period. The analysis results from the 156 samples using the method indicated that colored sand tracers were detected from samples of the primary observation in the range of up to distance of about 500 m from the location of the tracer injection. After 6 months passed, the tracer had been distributed throughout the survey area. Therefore, the sediment in the tidal flat was estimated to move widely but to remain in the tidal flat.

38: Four-year investigations into asari clam and molluscan community in tidal flats after the 2011 Tohoku earthquake

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The 2011 Tohoku earthquake and tsunami caused a lot of damage to the Tohoku region including marine life and fisheries industry. Bivalves like *Mya arenaria oonogai* normally distributed in deep and asari clam *Ruditapes philippinarum* were dug out to surface by liquefaction and by strong wave action of massive tsunami. The "tsunami break" was observed almost all shell surface of asari clam collected in May 2011. The earthquake caused sudden land subsidence of about 1m in maximum where intertidal zone became subtidal zone. Spat of oyster *Crassostrea gigas* was observed to attach to new hard substances after summer 2011. *Littorina brevicula* and *Batillaria cumingi* were found at shores that were land before the earthquake. What creatures advanced to new environment and disappeared within four years after the tsunami? The result suggests that sort of disturbance was different by place. The species richness decreased significantly at Matsukawa-ura Inlet, Fukushima, in comparison to other two sites investigated in this study. Larval recruitment of asari clam was found every year after the tsunami, and they are surviving and growing. Reed field were rushed by the tsunami, and mollusks that depend on reed also disappeared. Juveniles of deep burrowing bivalve *Macoma contacrata* have not developed after the earthquake. In Matsushima, Miyagi, juveniles and adults of mollusks were found just after the tsunami at the same place. The community structures have not changed since the earthquake. *Musculista senhousia* dominated in the place of subsidence in Mangoku-ura Inlet after the earthquake. From the result of nMDS analysis, molluscan community gradually changed in Mangoku-ura Inlet. Dominant species changed from species live in sandy bottom (asari clam) to those live in muddy bottom (*M. incongrua* and *C. angustus*). We have to clarify not only short-term effect which may explain an initial recovery of intertidal and subtidal animals but also a long-term effect which may explain continuous changes in population with land subsidence.

39: Effect of alien predator *Euspira fortunei* on the distribution and abundance of asari clam after the 2011 Tohoku earthquake and tsunami

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On 11 March 2011, the great earthquake occurred in Japan. It caused huge damage to the asari clam *Ruditapes philippinarum* along the Pacific coast of northern Japan. The alien naticid gastropod *Euspira fortune*, which was introduced with imported asari clams from China and Korea, prey on asari clam and other mollusks, has been found living after the earthquake. The purpose of this study is to examine the distribution and abundance of asari clam and *E. fortunei* after the earthquake. Quantitative and qualitative samplings were conducted in the tidal flats at Mangoku-ura Inlet and Matsushima Bay in Miyagi Prefecture and Matsukawa-ura Inlet in Fukushima Prefecture from May 2011 to November 2014. The size and weight were measured in all individuals collected. Population density of *E.*

fortunei dramatically decreased after the earthquake but many juveniles have collected in 2012. However, it has been decreased since 2013. Over 500 dead shells including about 150 shells with drill hole were collected from a quadrat of Matsukawa-ura Inlet in July 2011. *E. fortunei* ate at least 15 species of mollusks and diets of the snail were heavily dominated by the infaunal bivalves mainly of Asari clam. Therefore, *E. fortunei* has given multiple effects on asari clam stocks after the earthquake.

40: Asari clam lived in new intertidal flat after subsidence of the 2011 Tohoku earthquake

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Mangoku-ura Inlet in Miyagi Prefecture, northern Japan had a great catch of asari clam and had great fields of shell gathering. Our study area "Ohama" in Mangoku-ura Inlet was one of the famous fields of shell gathering. The earthquake on March 11, 2011 caused land subsidence, and the land has sunk by about 78 cm at this location where the intertidal zone became subtidal zone. After that event, the mussel *Musculista senhousia* has increased dramatically and formed mats over the sediment surface. The presence of these mats has altered the benthic habitat. The mats have led to siltation of the sediment and have resulted in hypoxia of water just above the seabed. The mussel mats have reduced the densities of asari clam, which suggested that Ohama has become unsuitable for habitation of asari clam. Parts of the land became intertidal zone. In May 2013, habitat of asari clam has been confirmed in the new tidal flats where the land sank after the earthquake. It indicated asari clam changed their habitat to the new tidal flats. This examination proceeded by quadrat method to find creatures and measured distance from the shoreline. Finally, what creatures advanced to the new environment, focusing on asari clams inhabit conditions and growth condition were discussed. It was found asari clam lives in landside up to 8 m from the shoreline at greater ebb. Although the sexual maturation and reproduction was also observed, almost all the asari clams in new tidal flat had rounded shell morphology, indicating that growth was bad. In addition, the spats (shell length: 1 – 2 mm) have been collected through the year and few middle-sized individuals (shell length: 10 – 20 mm). The recruitment and growth of asari clams were not constant by 2014. These suggested the new environment was not suitable for clam growth and survival. Since 2015, the number of spats and middle-sized individuals has increased. It suggested this new tidal flat is changing continuously.

41: Development of in situ measurement of clearance rate of the Manila clam under suspended culture conditions

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Due to drastically declining production of the Manila clam (*Ruditapes philippinarum*) fishery caused by environmental degradation and other anthropogenic effects, suspended culture of the Manila clam has recently been receiving much attention in Japan. Research institutes are trying to improve the efficiency of suspended culture to promote commercialization of this technique. Bivalve suspended cultures are commonly operated on sessile or epibenthic species, such as oysters and scallops that do not require sediments. In the case of the bottom burrowing Manila clam, however, the suspended culture is done in a container (e.g. plastic container or net cage) with substrate (e.g. sand, gravel or pumice) hanging in the water column. Manila clams in suspended culture thus face environmental conditions quite different from those in their natural habitat. In this study, a method for in situ water clearance measurement was developed

for monitoring physiological conditions of the Manila clam under suspended culture conditions. The device consisted of a semi-translucent plastic chamber (55 L capacity) with two smaller plastic boxes attached to both lateral sides. The smaller boxes were equipped with optical chlorophyll/turbidity loggers (Compact-CLW, JFE Advantech Co. Ltd.), and the inside of each box was painted a non-reflective matte black. One of the boxes had a submersible water pump, which pumped water out at a rate of 240 L/hr to the water surface via a tube, drawing in water from the other smaller box though the main chamber containing Manila clams. Preliminary test runs with (600 pieces of Manila clams, approx. 3 cm shell length) and without Mania clams in the chamber were carried out at a depth of 2 m, suspended from a pier at the National Research Institute of Aquaculture in Mie, Japan. Inflow and outflow chlorophyll *a* (chl *a*) concentrations were simultaneously measured every 15 minutes (i.e. complete water replacement in the chamber occurred in about 15 minutes). Water clearance rate (CR) was calculated as $CR = F [(C_i - C_0) / C_0]$, where F is flow rate, C_i is inflow chl *a* concentration and C_o is outflow chl *a* concentration. The mean C_i and C_o did not differ significantly without Manila clams ($p = 0.24$). The mean C_o (2.13 μg/L) was significantly lower than the mean C_i (3.04 μg/L) with Manila clams ($p < 0.001$). With Manila clams, the CR greatly fluctuated ranging from 0 to 616 mL/hr/ind, and the mean CR (178.1 ± 103.7 mL/hr/ind) was 10 to 20 times lower than previously reported values measured for similar sized clams at similar temperatures in the laboratory (i.e. in beakers). The CR changed gradually, and it seemed all the clams in the chamber were acting similarly. However, the pattern in the CR change was irregular and did not correlate with tidal height, water temperature or time of the day. The CR was positively correlated with C_i ($p < 0.001$).

42: Growth and food environment of the Manila clam in suspended culture

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Suspended culture of the Manila clam (*Ruditapes philippinarum*) has recently been developed due to drastic decline of the fishery production. In this study, we compered the growth of the clam between suspend culture in a plastic container in the water column and bottom culture in a mesh bag in a tidal flat. We also determined the relationship between the growth of the clam and food environment by analyzing chlorophyll *a* level and carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of the clam and particulate organic matter in water column and sediment. We conducted an experimental suspended culture of the clam at rafts in Gokasho Bay and Ounoura Bay in Mie from September 2012 to May 2013 and from March to September 2013. We suspended the clam in a plastic container with gravel at a depth of 2 m from the rafts, and cultured the clam in a mesh bag containing gravel placed in a tidal flat adjacent to the rafts. We measured the size, total wet weight and stable isotope ratios of the clam every one to four weeks, and also monitored the chlorophyll *a* level continuously using a logger. The fastest growth was observed in the suspended culture in Gokasho Bay, where the clam grew from 12.8 mm shell length (0.3 g) to 34.5 mm (8.2 g) in 8 months. The growth was faster in suspended culture than bottom culture by 2 to 4 times in shell length and 2 to 6 times in wet weight. The maximum mean growth rate was 0.26 mm/day (0.055 g/day) in suspended culture, and there was a significant positive correlation between the mean growth rate and chlorophyll *a* level. The stable isotopic signature indicated that the clam with faster growth mainly assimilated organic matter in the water column rather than those in the sediment.

43: Relationship between abundance of planktonic larvae and benthic juveniles of asari *Ruditapes philippinarum* in eastern Hokkaido, Japan

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The larval recruitment process has been focused as a key aspect for understanding the population dynamics of asari and improving stock enhancement strategies of this symbolic fishery species in coastal areas of Japan. In southern parts of Japan, such as Honshu and Kyusyu islands, asari usually spawn intermittently from spring to autumn, and thus multiple cohorts occur in the same year. In eastern Hokkaido contrastingly, asari show a single synchronized spawning event during summer because of a slow maturation process associated with low-temperature water from the cold Oyashio current. Additionally, they are distributed in semi-closed estuaries with minor larval exchanges among one another. Therefore, the population structures are simple, and this makes it suitable to trace each population and evaluate the dynamics during the early life stages including planktonic larvae and benthic juveniles. A research (2012 – 2015) on the planktonic larvae and benthic juveniles of asari in Akkeshi area consisting of estuary and bay, which is one of the main asari production area in eastern Hokkaido, showed that abundance and the appearance frequency of planktonic larvae did not have a clear relationship with the magnitude of recruitment of benthic juveniles to the intertidal fishing grounds in the estuary. Moreover, high level of settlement of benthic juveniles was observed at the fishing grounds only in 2012, wherein planktonic larvae were collected only once during the 7 sampling days only in the bay area. These results showed that a large number of the planktonic larvae in the estuary drifted to the bay and then succeeded in returning to the benthic habitats in the estuary only in 2012. This may explain the highly variable year class strength of asari in eastern Hokkaido.

This study was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Coastal Ecosystem Complex Project).

44: Energy budget and neuroenzymatic activity in asari, *Ruditapes philippinarum*, following short-term exposure to the toxic alga, *Heterocapsa circularisquama*: implications for reproductive success

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The Manila (asari) clam, *Ruditapes philippinarum*, is an important fishery resource in Japan; however, several factors have contributed to the decline of its commercial fishery production over the past two decades. Reclamation of tidal flats, overfishing, hypoxic coastal waters and diminished food supply as a result of eutrophication, heavy metal pollution, outbreaks of flood events, strong winds, the introduction of alien species including predators, and inadequate fishery management practices have all been identified as playing a major or a synergistic role in the decrease of Manila clam stocks. Pathogens, mainly bacterial and protozoans are also responsible for mass mortalities or the reduction of fitness of Manila clams so should do harmful algal blooms (HAB). Among the HAB occurring in Japan, *Heterocapsa circularisquama* is by far the most toxic to Manila clams, and to shellfish in general. Mass mortalities caused by this HAB are well-documented, recurrent, and geographically-expanding events around Japan. In spite of the relative decrease in the frequency and intensity of its blooms after 2000, *H. circularisquama* is highly lethal and/or detrimental even at low cell-density, and has been going through a revival period since 2008. The mortalities of Manila clams in fishing grounds have been reported when *H. circularisquama* blooms at several hundred cells

per ml. Nonetheless, the impacts of background exposures to *H. circularisquama* on Manila clams, and bivalves in general, have so far been overlooked. Therefore, we assessed the energy budget and neuroenzymatic activity of adult Manila clams following short-term exposures to several realistic cell densities of *H. circularisquama*, at two incubation temperatures which are lower than the ones at which *H. circularisquama* causes the reported mortality in Asari. The Energy budget (J h^{-1} g^{-1}) was significantly affected following a 2-h exposure to 50 and 5 cells ml^{-1} *H. circularisquama* at 15 and 20°C, respectively. The Acetylcholinesterase activity (nmol min^{-1} mg total $protein^{-1}$) was also decreased following 3 – 48 h of exposure to 5 cells ml^{-1}. The results of this study demonstrate that even short exposures to low cell densities of *H. circularisquama* could affect the key neuroenzymatic activity acetylcholine and the energetic performance of Manila clams without necessarily causing mortalities. These findings imply that *H. circularisquama* may play a role not only in the survivability of Manila clam fishing grounds but also on the reproductive success of Manila clam in Japan. The management and restoration of sustainable fishing grounds of Manila clam would reply on proper understanding of the environmental factors that contribute the most to stock depletion, which should include HAB as background negative effectors.

45: Evaluating the growth and mortality of transplanted asari *Ruditapes philippinarum* juveniles in the Matsusaka region, Mie prefecture, Japan

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Asari juveniles settle in estuary and often die out due to freshwater discharges, resulting in failed recruitment into commercial size (> 20 mm) in the Matsunase area Mie, Japan. In this study, we examined the growth and mortality of asari juveniles transplanted to two subtidal areas (Matsunase area: M1, Miwatari area: M2) with environments different from those in the nearby estuary areas to simultaneously monitor physical environments (i.e., wave-current flow, salinity, temperature, dissolved oxygen and turbidity) above the bottom sediment. Initial density of transplanted asari juveniles was approximately 1200 ind/m^2 in both areas on 31 May 2013. The density of transplanted asari juveniles at M1 drastically decreased during the first two months to 35 ind/m^2 on 8 July and then to 2 ind/m^2 on 19 August 2013. In contrast, the density at M2 was 534 ind/m^2 on 8 July, 262 ind/m^2 on 10 September and 120 ind/m^2 on 2 October 2013. We also examined the characteristics of mortality of transplanted asari juveniles in relation to physical environments, using generalized liner model (GLM). A best-fitted model selected the maximum wave-current velocity during the survey period as the positive explanatory variable for the juvenile growth although the velocity at M1 tended to be higher than at M2 that had higher juvenile survival than at M1. Thus, growth and survival are in trade-off relationship, and in order to maximize the efficiency of transplantation, a habitat with lower wave current velocity such as M2 should be selected.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

46: Risk assessment on the mortality of asari clam under oxygen deficiency

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Oxygen deficiency has often occurred in major habitats of asari clam in Japan, such as Tokyo Bay, Mikawa Bay, Ise Bay, and Isahaya Bay. It has been considered that prolonged oxygen deficiency brought about the mass mortality of the clam. On the other hands, most bivalves can switch to anaerobiosis to maintain their metabolism and survive several days in anoxic conditions. Asari clam also has tolerance for anoxic and hypoxic conditions through the anaerobic biosis. Since the impact of anoxia on the clam population is poorly understood, it is necessary to quantitatively assess the risk of oxygen deficiency for survival of the clam. In order to evaluate the effect of low oxygen concentration on the survival of asari clam, exposure experiments at different oxygen concentrations (1.0 mg/l, 0.5 mg/l, 0 mg/l and 0mg/l with 10 mg-S/l sodium sulfide) were conducted in 700 ml volume containers. Ten clams were placed in each container and water temperature was set at 20, 25 and 30°C. The results showed that mortality was higher at lower oxygen levels and with the presence of sulfide. We obtained the following linear regression curves to express the median lethal time (LT_{50}, h) as a function of water temperature (WT, °C). Oxygen concentration of 1.0 mg/l: $LT_{50} = -17.7\ WT + 582.7$ ($R^2 = 1$), 0.5 mg/l: $LT_{50} = -15.7\ WT + 523.9$ ($R^2 = 1$), Anoxic: $LT_{50} = -9.4\ WT + 318.4$ ($R^2 = 0.98$), Anoxic + Na_2S: $LT_{50} = -3.2\ WT + 129.2$ ($R^2 = 0.72$). Particularly, the mortality rate (Mo, %) in the anoxic condition was expressed as a multiple regression equation with WT and duration of anoxia (D). $Mo = 26.3\ WT + 2.6\ D - 828.6$. We further conducted experiments for evaluating the long-term influence of experienced anoxia on the survival of the clams. Asari clams were exposed to anoxic water for 24, 48, and 72 h at 20 and 25°C. After the exposure, the clams were placed in oxygen-saturated water, and the survival of the clams was subsequently monitored for two weeks. The mortality rate in two weeks (Mo') was expressed as the following equation. $Mo' = 6.0\ WT + 1.9\ D - 167.6$. The results implied that anoxia caused not only acute but more seriously prolonged mortality. The equations would be useful for predicting the mortality in combination with the in situ measurement of oxygen deficiency.

Workshop on the Manila (asari) clam

The third international symposium on Manila (asari) clam was followed by a workshop to share and comparatively analyze information on the trends of asari fishery and aquaculture production from major producing countries at the National Research Institute of Aquaculture, Fisheries Research Agency in Minamiise, Mie, Japan, hosted by the same institute on June 3, 2015.

Brief country reports under a fixed format were presented by the participants from Canada, China, France, Italy, Japan, Korea, Portugal and Turkey to understand the differences and commonalities of the production methods, social, economical and biological aspects in each country. Major constraints for the clam production and possible countermeasures for the recovery of the production were discussed. Furthermore, based on the discussion, possibilities of future research collaborations were sought out among the participating countries. A framework for mutual information exchange was also established.

Aside from the major points discussed, one of the proposals raised in the workshop was standardization of condition index (i.e. weight to size ratio) of asari. Different researchers use different condition index (condition factor), making it difficult to compare data among reports. The participants agreed to try to resolve this issue.

Program

June 3, 2015 (Wednesday)

Venue: National Research Institute of Aquaculture, Fisheries Research Agency, Nakatsuhamaura, Minamiise, Mie, Japan

09 : 00–9 : 05: Opening Remarks
Megumi Minagawa (Director General, National Research Institute of Aquaculture, Fisheries Research Agency)

09 : 05–9 : 15: Purpose, objectives, and organization for the workshop
Junya Higano, (National Research Institute of Aquaculture, Fisheries Research Agency)

09 : 15–10 : 30: Country report presentations
Canada: Brian Kingzett (Vancouver Island University)
China: Fang Jian-Guang (Yellow Sea Fisheries Research Institute)
France: Xavier de Montaudouin (University of Bordeaux)
Italy: Marco Bartoli (Parma University)
Japan: Natsuki Hasegawa (Fisheries Research Agency)
Korea: Kwan-Sik Choi(Jeju National University)
Portugal: Stefania Chiesa (University of Aveiro)
Turkey: Serpil Sarder (Ege University)

10 : 30–10 : 40: Coffee break

10 : 40–12 : 00: General Discussion

12 : 00–12 : 10: Closing remarks
Kwan-Sik Choi (Jeju National University)

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Country report from Canada

Brian KINGZETT*1

General remarks on the Manila clam fishery

Landings of Manila clams and mixed steamers (which are presumed to be primarily Manila clams) increased dramatically in the early 1980's, averaging 1,654 t between 1980 and 2000, as opposed to 189 t from 1951 to 1979. Manila clam landings decreased after 1988, primarily as a result of more restrictive management measures. In recent years, openings have been reduced in most areas to only a few days per year. Regardless of reduced opportunity, landings increased to 1,327 t in 1994, and have fluctuated between approximately 1,100 and 1,500 t since then. However, these statistics include depuration landings since 1994, representing the revival of operations on some beaches that had been lost to contamination.

Licensed commercial clam diggers are required to designate one of six clam areas they wish to fish each year and then compete for available clams during short intense openings. In 1998, the intertidal clam fishery underwent license limitations, resulting in a reduction from approximately 2,500 licensed diggers to current levels of 450 Z2 and 579 Z2ACL (Aboriginal Commercial License) license holders. Z2ACL licenses are held by various First Nations (aboriginal groups), which subsequently distribute them to members. Z2 and Z2ACL license holders are required to renew commercial clam licenses annually. In addition to the commercial clam licenses, the holder is required to be registered as a commercial fish harvester and have a Fisher's Registration Card (FRC).

A recreational fishery occurs coast-wide where areas are open for harvest. A British Columbia Tidal Waters Sport Fishing License is required for the recreational harvest of all species of fish including shellfish. Daily limit is 75 clams/person.

First Nations' harvest for Food Social and Ceremonial (traditional) purposes may occur coast-wide where areas are open for harvest. The number of First Nation harvesters and quantity is unknown.

The depuration fishery is part of the commercial intertidal clam fishery, conducted under specific licenses at registered Depuration Plants. Access to beaches requires stock assessment, notification and reporting requirements that are different than the commercial competitive fishery and often occur during times when there are no clam harvest openings.

Basic information on fishery

Geography and geomorphological characteristics: Manila clam fishing is mainly operated in tidal flats in sand/mud and small gravel substrates.

Fishing method: Manual harvest with rakes, some implementation and testing of automatic harvesting machines

Fishery management: Shell size regulations 38 mm (wild Fishery only), Short Open periods within fishing zones. Growing water classification and management through the Canadian Shellfish Sanitation Program

Standing stock assessment: Statistical survey by local governments and fisherman's associations

Basic information on aquaculture

Environmental characteristics: Manila clams are found in mid intertidal mixes of sand/mud/" pea" gravel substrates.

Culture methods: Bottom culture by reseeding using hatchery produced seed and mix of wild

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recruitment. Lightweight mesh netting is placed on clam beds to reduce predation by ducks, fish and invertebrates. Suspended cage culture has been tried in the past but abandoned due to persistence of poor shell shape and high mortalities.

Operational management: Intertidal clams are also accessed commercially through aquaculture operations. These are managed independent of the wild commercial fishery. Farming operations operated on intertidal lands leased from the Provincial government for terms of 10 - 30 years. Licensing of Aquaculture is conducted by the Federal Government.

Others: Obtaining new areas for aquaculture is very difficult at present. Aboriginal Groups (First Nations) have most significant access to new growing areas as part of treaty process. As a result, significant increases in production are not anticipated.

Major constraints and countermeasures

Habitat degradation: Many optimal beaches are closed to harvest due to sewage contamination.

Overfishing: Strict license limitations, increased production from aquaculture and fact that clams may only be sold to federally licensed processor with chain of custody have reduced overfishing. Poaching from contaminated areas is still a concern.

Diseases and parasites: Not seen as limiting.

Climate change: Increasing temperatures in culture region may have effect on wild recruitment.

Economic aspects: Prices have decreased in recent years but are relatively stable at present. Aging workforce will require mechanization for future development.

Others: The use of clam netting on beaches fronting residential areas has become controversial with public opposition in some areas. Use of mechanical harvesters has also been opposed for reasons of industrialization of the foreshore.

Proposed international collaborative studies in the future: Improvements in nursery culture.

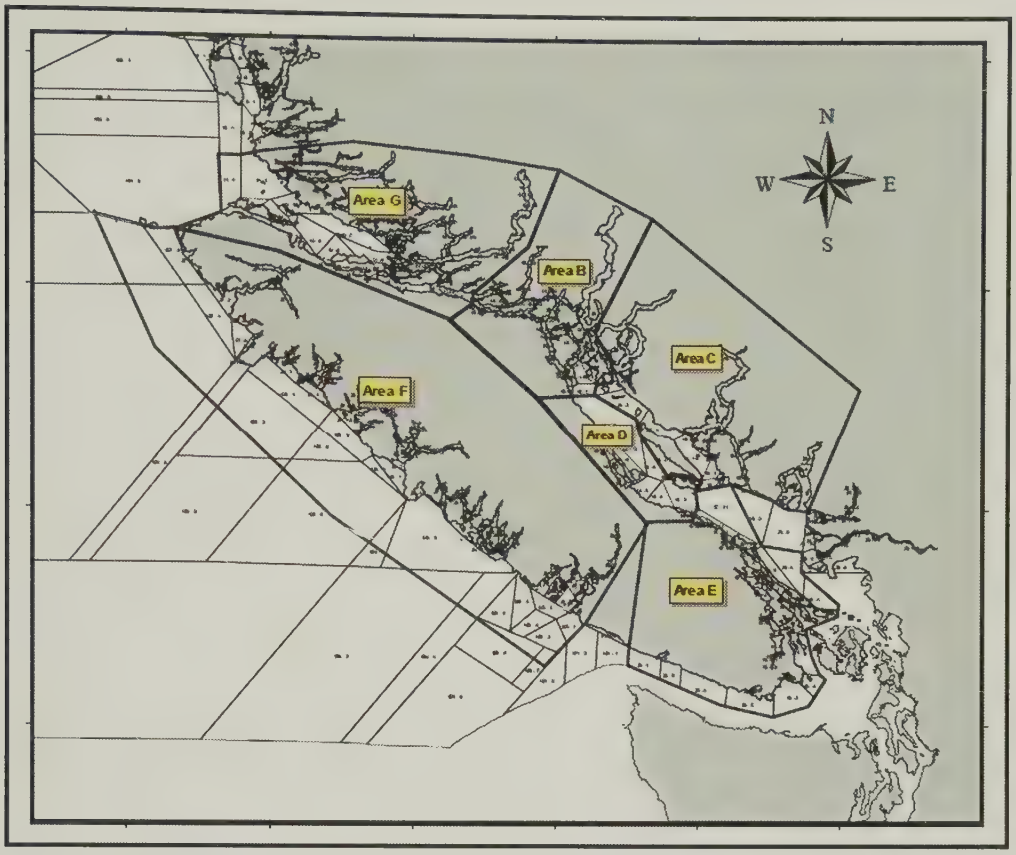

Fig. 1. Fishery management areas in Southern British Columbia, Canada

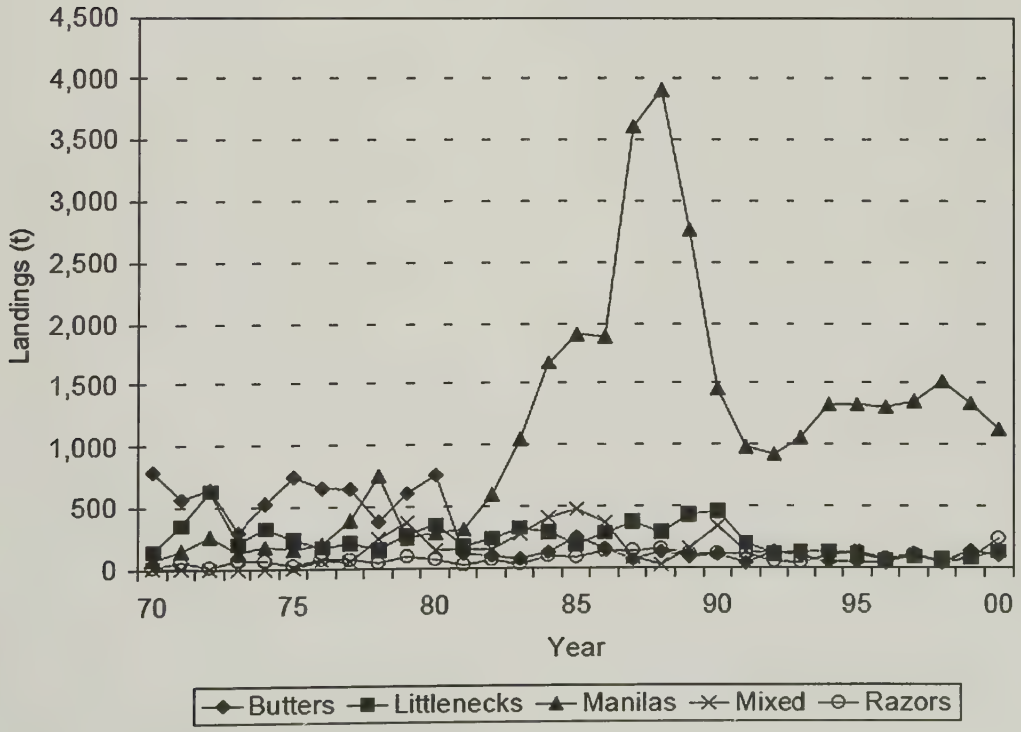

Fig. 2. Fisheries Landings for Intertidal Clams in British Columbia, Canada

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Country report from China

Jianguang FANG*1

General remarks on the Manila clam fishery

Over 2 million tons of Manila clams *Ruditapes philippinarum* (Adams et Reeve) were produced each year on the coast of Yellow Sea, Bohai Sea and East China Sea of China. Most of this production occurs in Liaoning, Shandong and Fujian Provinces. In addition, a significant Manila clam seed production industry has developed with pond production facilities in Fujian province. Before 1970's, all farmed seeds of Manila clam relied on natural. Farmers collected seed of Manila clam from natural inhabit. By the late of 1970's, the seed production technology of Manila clam in ponds was developed rapidly and achieved great success in Fujian Provinces because the natural seed could not enough to meet the demand from farming.

Since 1990's, the Manila clam industry has developed rapidly in China prompted by the demand from domestic and abroad. In order to meet the increasing demand of seed from whole China, the seed production of Manila clam in reclamation areas was became the major sources of seed of manila clam for farming in China. In 2013, the total seed production in Fujian province was 7952 tones with shell length about 1cm. Meanwhile, the Manila clam is also the major species farmed with shrimp, fish, crab in form of pond integrated multi-trophic aquaculture, which is practiced in commercial scale in Zhejiang and Fujian Provinces.

In history, the farming of Manila clam was mainly carried out in tidal zone and subtidal zone before 1980's. An unfilled domestic and overseas demand was pushing attempts to increase production of this clam from 1990's. The farming areas of the clam has extended from intertidal zone to shallow sea with the depth of about 10 m in Shandong and Liaoning provinces. Because of longer time immersing in water and richer supply of phytoplankton, the growth rate and suitable harvest time of the clam cultivated in deeper water is much higher and longer than that of clam growing in natural inhabits. The main problems for such farming are the predation from crab, starfish and sea snails.

The farming space decreasing mainly due to sea reclamation along the coast and the pollution from lands are being the major threaten to Manila clam industry in China

Basic information on fishery

Geography and geomorphological characteristics: Manila clam fishing is mainly operated in tidal flats and shallow waters in calm inner bay with sandy and sand-muddy habitat.
Fishing method: Short shaft raking in tidal flat, long shaft raking in subtidal water, clam dredge and hydraulic dredge in deeper water.
Fishery management: Fishing closed season from June 1 to August 31.
Standing stock assessment: Statistical survey by local governments and fisherman's associations.

Basic information on aquaculture

Environmental characteristics: Aquaculture of the Manila clam is mostly done in tidal flats, subtidal zone and deeper waters (<10 m) in China.
Culture methods: Bottom culture is the main farming method by reseeding natural juveniles, and artificial seeds produced in Fujian Province. Pond culture is being developed recently with Integrated Multi-trophic Aquaculture models in Zhejiang and Fujian Provinces.

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Operational management: In some intensive farming areas, such as Jiaozhou Bay in Qingdao, the rotate culture patterns was adapted in order to control the total production is lower than carrying capacity.

Major constraints and countermeasures

Habitat degradation: Habitat area of the Manila clam significantly decreased by reclamation in the last decade along the coastline of China. Dam construction caused the change of current, bottom substrata directly or indirectly. Eutrophication caused by human activities was another harmful stress to habitats of Manila clam.

Overfishing: Over catching has destroyed the clam population in many places since the 1980s. It seemed to be the most possible cause for rapid decrease of the clam production in Shandong, Liaoning and Fujian Provinces. Nowadays, there is almost no natural Manila clam fishing.

Diseases and parasites: No great attention is paid on prevalence of diseases and parasites on Manila clam. Abrupt mortality occurred in summer, especially occurred after heavy storm has recorded in Jiaozhou Bay, Qingdao recent years.

Climate change: Change of food environment is considered to be associated with acidification.

Economic aspects: Relatively low farm gate price.

Proposed international collaborative studies in the future: Natural habitat recovery and improvement for Manila clam. Influence of Acidification on development and growth of Manila clam.

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Country report from France

Xavier de MONTAUDOUIN*1

General remarks on the Manila clam fishery

In 1972, France underwent an oyster (*Crassostrea angulata*) crisis and urgently needed to diversify its aquaculture. Thus, Asari clam (*Ruditapes philippinarum*) was introduced at that time for aquaculture purpose, concomitantly with the introduction of Pacific oyster (*Crassostrea gigas*). All Asari clam adults and spat originated from the same area (Puget Sound, WA, USA). After a promising start and the implementation of a national Research & Development program, Asari clam culture rapidly faced a series of concomitant handicaps: firstly, in spite the fact that cultural practices were optimized and locking points addressed, leasing ground availability was limited during the 1980s' due to certain reluctance from oyster farmers to share their leases and/or diversify their activity; secondly, mortality events in the parks probably due to the spat quality, diseases and/or zootechnical errors and/or predation by triggerfish; and thirdly economical competition with Italian production rapidly increased, exacerbated by the occurrence of neonaturalized Asari clam populations and the resulting professional fishing. Meanwhile, European vs national regulations, concerning minimum legal shell length of clams devoted to the market, were unfavourable to France due to contrasted rules between the Mediterranean Sea and the Atlantic Ocean; eventually, several diseases impacted drastically clam populations. At the end of the 80's in Northern part of Brittany (France), mass mortality occurred due to brown ring disease, later related to a prokaryote (*Vibrio tapetis*).

Presently, the French production remains limited to 2 - 3000 metric tons, mainly based upon professional fishing on neonaturalized populations located in two sites (Arcachon bay and Morbihan Gulf). In Arcachon bay, a comprehensive population dynamics study demonstrated the concomitant effects of fishing activity and environmental characteristics on the population dynamics. A management model was developed to assess various scenarios mainly based on conservation measures (*i.e.* fishing area, and/or fishing licences number, and/or fishing period). Implementation of those recommendations has provided some encouraging results. However, Asari clam fitness remains poor: a genetic impoverishment due to population isolation was argued by fishermen, but transplant experiments demonstrated that these bivalves kept their plasticity, at least in terms of growth and condition index. Thus, several environmental factors were investigated as possible key parameters explaining low clam performances. Again, pathologies were pointed out and particularly the high pressure exerted by the protist *Perkinsus* sp. (perkinsosis). Moreover, a new pathology was discovered in 2005 in Arcachon bay, the brown muscle disease. Although the etiological factor has not been confirmed, viral origin is suspected.

A meta-analysis comparing Asari clam characteristics in Arcachon Bay with the international literature pointed out that their reduced condition index in this bay was likely resulting from combined unfavorable factors (e.g., diseases, trace elements). However, 30% of the condition index variability among sites at the worldwide level was explained by food availability (chlorophyll *a* concentration). A comparative morphometric studies on four populations of the French Atlantic coasts, using conventional shape analysis, also revealed significant relationships between morphometric ratios and environmental parameters (chlorophyll *a*

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concentrations and seawater temperature).

Eventually, marine ecologists and state managers as well, must deal with conflictual goals: on one hand Asari clam is an important exploited bivalve in France, on the other hand, the species remains an exotic species which needs careful attention (e.g., European regulations on invasive species).

Basic information on fishery

Geography and geomorphological characteristics: Asari clam fishing is mainly operated on muddy tidal flats, often covered by *Zostera* beds.

Fishing method: By hand, clam dredge, hydraulic dredge

Fishery management: Shell size regulations, fishing closed season, fishing areas, limited licenses

Standing stock assessment: Statistical survey by local fisherman's associations with scientific assistance: not exhaustive (few areas are investigated)

Basic information on aquaculture

Environmental characteristics: Most of the production is from fishery. Asari clam is mostly done on tidal flats.

Culture methods: Smaller part of the national production is bottom culture by seeding, using spats from hatchery.

Major constraints and countermeasures

Habitat degradation: No, except seagrass (Asari clam habitat) that is declining.

Overfishing: Not obvious.

Diseases and parasites: Yes, Large prevalence of *Perkinsus olseni* and rather high intensity. *Vibrio tapetis* is still occurring in the north of the country while brown muscle disease is endemic of Arcachon Bay.

Climate change: Change of food environment is considered to be associated with sea level rise.

Economic aspects: Relatively low price despite low production. Rough competition within Europe.

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Country report from Italy

Marco BARTOLI*1

General remarks on the Manila clam fishery

The asari clam was introduced in Italy in 1986 and became rapidly one of the most important economic activity within national aquaculture product. The introduction of the asari clam in Italy is a quite cited example of successful introduction of an exotic species, contrasting ecological theories. Main sites of production are the north eastern italian lagoons of Marano-Grado, Venice and those within the Po River Delta (Fig. 1). Since its introduction, the production peaked exponentially, reaching in 1999 some 65,000 tons; at present the national production is nearly 35,000 tons in 2013 (Fig. 2). The main reason for such decrease is the much lower contribution of the Venice lagoon to the total, as a consequence of multiple factors (oligotrophication of the lagoon, anoxia, parasites, bad management of the resource). Elsewhere (Goro Lagoon, the study case presented) the production remained stable or even tended to increase due to appropriate management practices, targeting the tidal canals, the substratum and the creation of a nursery area.

Basic information on fishery

Geography and geomorphological characteristics: Asari clam fishing is mainly operated in sandy microtidal flats and shallow sub-tidal waters in semi-enclosed lagoons.
Fishing method: Short shaft raking, long shaft raking, clam dredge, hydraulic dredge (the latter most used at present).
Fishery management: Shell size regulations, fishing closed season, joint selling via cooperatives.
Standing stock assessment: Statistical survey by fisherman's associations.
Others: Special attention on natural nursery areas outside lagoons protected by events as anoxia or macroalgal blooms.

Basic information on aquaculture

Environmental characteristics: Aquaculture of the asari clam is mostly done on fishing grounds in microtidal flats (sandy and muddy sandy substrate) in Italy.
Culture methods: Bottom culture by reseeding using transplanted natural spats.
Operational management: Frequent cleaning of farming areas and removal of opportunistic macroalgae.
Others: Special attention to sustainable farming densities (max 500 ind/m^2) in order to avoid oxygen depletion and sulfide production, as well as algal blooms (mathematical models specifically developed).

Major constraints and countermeasures

Habitat degradation: This is a consequence of harvesting techniques (destruction of natural sediment gradients, re-suspension of nutrients, drastic drop of oxygen due to chemical oxidation of reduced compounds), increase of sedimentary oxygen demand, large macroalgal blooms. Frequent movements of sands from marine to brackish areas, frequent dredging of tidal canals to improve water circulation, frequent harvesting of macroalgae.
Overfishing: This has happened in the Venice lagoon and in part in the Goro Lagoon. In the latter fishermen associations decided to invest in nursery areas in the open sea where clams are not collected and constitute a "buffer" for period of crisis, providing seeds.

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Diseases and parasites: Not relevant for the stocks

Climate change: There are not demonstrated effects up to now. Changing river flow may affect freshwater transport and lagoon salinities with some impacts. However, these are not relevant at the moment.

Economic aspects: Wide variations of prices due to unregulated marked (very beginning). At present the prices are relatively low (2 – 4 euros per kg) but stable.

Others: Predation by crabs if seeded clams are too small.

Proposed international collaborative studies in the future: Very welcome. The Sacca di Goro Lagoon has been a key study site in different National and European projects targeting impacts (anoxia, macroalgae) and sustainability (economic and environmental).

Fig. 1. Main sites of farming of Ruditapes philippinarum in northeastern part Italy.

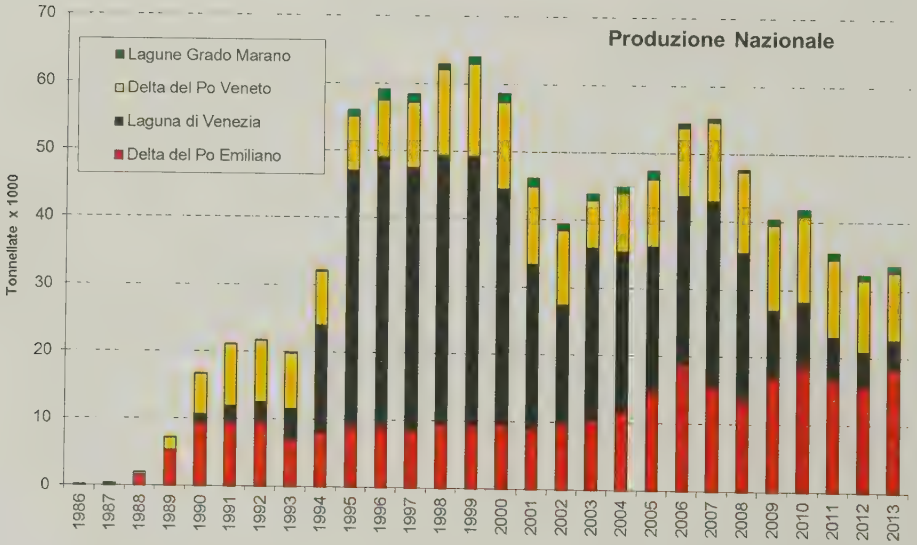

Fig. 2. Annual production (1986 – 2013) of Ruditapes philippinarum in the main production sites in Italy. The crisis of the production in the Venice lagoon is evident (black bar).

水研機構研報, 第42号, 117－118, 平成28年
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Country report from Japan

Natsuki HASEGAWA*1, Satoshi WATANABE*1, Junya HIGANO*1, and Mitsuharu TOBA*2

General remarks on the Manila clam fishery

Asari is one of the most important fisheries species in the coastal waters of Japan. The production of the asari fishery in Chiba prefecture had occupied more than half of the national production in early 1960's. Rampant land reclamation from 1960's to 1970's, however, sevierly reduced the wild stock of asari in Chiba, and the reduction was compensated by increased production in Kumamoto prefecture, which had the largest tidal flat area in Japan. The annual production was more than 100 thousand tons from 1960's to mid 1980's. However, constant decrease of the production in Kumamoto has resulted in reduction in the national production since 1980s. Only the production in Aichi Prefecture has been maintained relatively constant for about 50 years. The national production was about 22,700 tons in 2013 around 15% of the peak value in 1980's. The reduction of the wild stock is attributed to many factors such as reclamation, reduced sand supply by dams, sediment siltation, hypoxia, redtide, predation, parasite infection and disease. The production of the clam in Japan mostly depends on capture fisheries.

Basic information on fishery

Geography and geomorphological characteristics: Asari fishing is mainly operated in sandy tidal flats and shallow sub-tidal waters in calm inner bay.
Fishing method: Short shaft raking, long shaft raking, clam dredge, hydraulic dredge.
Fishery management: Shell size regulations, fishing closed season and joint selling.
Standing stock assessment: Statistical survey by local governments and fisheries cooperative associations.

Basic information on aquaculture

Environmental characteristics: Aquaculture of asari is mostly done on fishing grounds in tidal flats in Japan.
Culture methods: Bottom culture by reseeding using transplanted natural spats. Suspended cage culture is being developed and recently getting much attention.

Major constraints and countermeasures

Habitat degradation: Habitat area of asari significantly decreased by reclamation in 1960s in Tokyo Bay. Dam construction caused the change of bottom substrata directly or indirectly. Anoxic or hypoxic water in Ariake Sound, Mikawa Bay, Ise Bay, Tokyo Bay caused by phytoplankton blooms.
Overfishing: Over catching destroyed the clam population in many places in 1980s and 1990s. It seemed to be the largest cause for rapid decrease of the clam production in Kumamoto, Fukuoka and Oita prefectures. Fear for the over catching is still present to some extent.
Diseases and parasites: Broad prevalence of *Parkinsus honshuensis*, *Vibrio tapetis*, abrupt occurrence of a parasitic sea spider, *Nymphonella tapetis* in Kisarazu in Tokyo Bay.
Climate change: Change of food environment is considered to be associated with sea level rise.
Economic aspects: Relatively low farm gate price despite low production.
Others: Heavy predation by eagle ray, *Aetobatus narutobiei* and the alien naticid gastropod *Euspira fortunei* has been occurring in Western and Northern part of Japan, respectively.
Proposed international collaborative studies in

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the future: Creation of international researcher's networks on asari.

Fig. 1. Main bay and estuary habitats of *Ruditapes philippinarum* and other filter feeding bivalves in Japan.

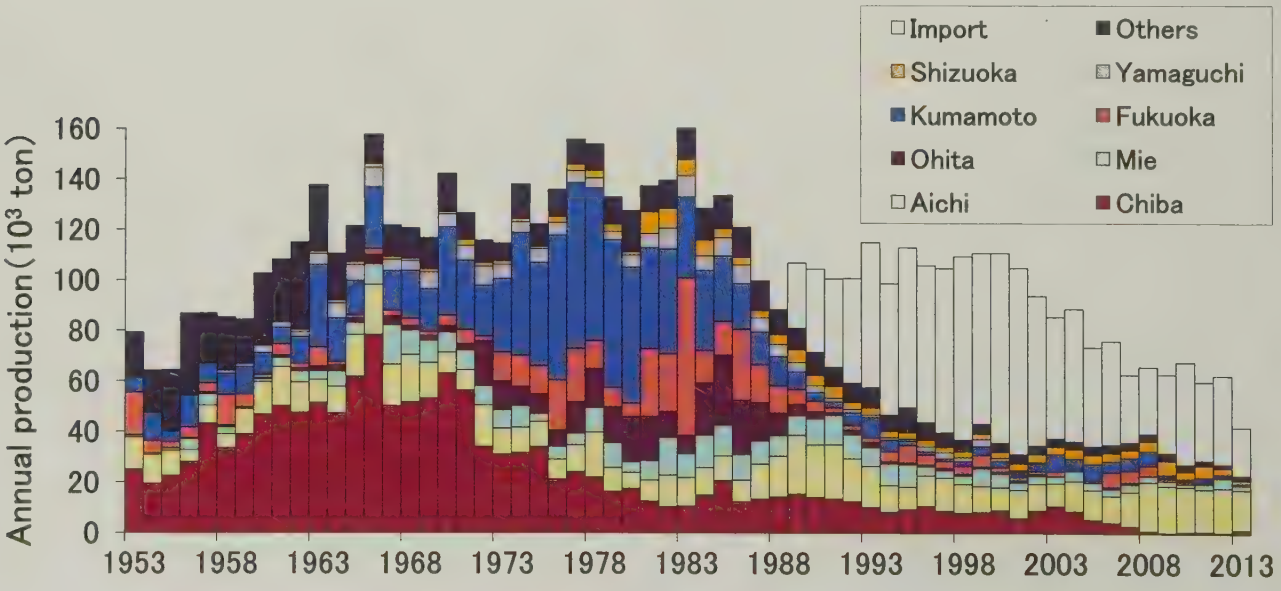

Fig. 2. Annual production and imported amount of *Ruditapes philippinarum* in Japan.

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Country report from South Korea

Kyung-Il PARK*1 and Kwang-Sik CHOI*2

General remarks on the Manila clam fishery

Manila clams, along with oysters and mussels, represent one of the most important bivalve fishery resources in Korea. Manila clam production is largely divided into coastal aquaculture and marine fishery. In coastal aquaculture, clam seeds are planted in tidal flats, and once they mature into adults, they are harvested. In marine fishery, wild Manila clams living in subtidal zones are harvested using fishing dredges. Clam aquaculture is developed in the western coast because tidal flats are well formed, whereas marine fishery of Manila clams has been predominant in the southern coast because it has a well-developed ria coast. Although Manila clam production in South Korea amounted to only 10,000 tonnes in the early 1970s, it increased rapidly to over 80,000 tonnes by the end of the 1980s, because of continued advances in aquaculture methods. However, the production started to decrease rapidly from the early 1990s, and dropped to approximately 30,000 tonnes by the middle of 1990s, with this trend continuing until the early 2010s. In 2014, Manila clam production was only about 20,000 tonnes after continued decline in production. This rapid decline in Manila clam production was caused by the rapid decrease in coastal aquaculture production rather than the decreased yield from marine fishery. The decline in Manila clam production from coastal aquaculture is attributed to low yields from the provinces of Jeonbuk, Gyeonggi, and Jeonnam, which have been the major producers of clam through coastal culture. It has been speculated that the rapid decline in Manila clam production in these areas were caused by the reduction in habitat areas due to tidal flat reclamation, which caused reduction in development areas for young clams and mass mortality during the spring and summer seasons. This decline in production is causing serious shortage of supply, leading to the import of Manila clams for meeting the consumer demand. From 2008 to 2014, 30,000–40,000 tonnes of live Manila clams have been imported each year, which exceeds the total Manila clam production in Korea. Furthermore, import of Manila clams include not only adult clams for direct consumption, but also young bivalves; in the 2010s, approximately 5,000 tonnes of young bivalves were imported in Korea. Hence, part of the Manila clams produced in Korea by coastal aquaculture are from the imported clam seeds.

Basic information on fishery

Geography and geomorphological characteristics: Manila clam fishing is mainly operated in sandy tidal flats and shallow sub-tidal waters.
Fishing method: Clam digging, clam dredge.
Fishery management: No regulation.
Standing stock assessment: Statistical survey by local governments and fishermen's associations

Basic information on aquaculture

Environmental characteristics: Aquaculture of the Manila clams is mostly conducted on fishing grounds in tidal flats of Korea.
Culture methods: The most commonly used culture method is aquaculture: natural Manila clam seeds or imported clam seeds from China are planted in tidal flats, and once they mature into adults, they are harvested.
Operational management: Fishing village cooperatives have been established in each area of tidal flats to manage production, culture, and sale of

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Manila clams.

Major constraints and countermeasures

Habitat degradation: The area of tidal flats in South Korea totaled 3,203.5 km^2 in 1987, which, according to a survey in 2008, has reduced by 22.3% to 2,489.4 km^2. This reduction in tidal flat areas has caused reduction in Manila clam habitats.

Overfishing: Although overfishing is believed to have caused reduction in Manila clam brood stock, which has affected recruitment of clams, there have been no studies on this aspect.

Diseases and parasites: Most Manila clams inhabiting the tidal flats of the western coast have been infected with *Perkinsus olseni*, from which serious inflammatory responses to the infection have been observed. Although some Manila clams from the western and parts of the southern coasts have shown low infection rates, they have been infected with brown ring disease (*Vibrio tapetis*). Infection by *Marteilioides* sp., a species of Paramyxean parasites, along with *Cercaria tapidis*, *C. tapes*, and *Parvatrema timondavidi*, types of trematodes, have also been reported. Recently, mud shrimps (*Upogebia major*) have multiplied in great numbers in the habitats of Manila clams in the tidal flats of the western coast, which inhibit Manila clams from burrowing the substratum, leading to the decline in Manila clam population due to failure in habitat competition.

Climate change: Although a report speculated that the decline in Manila clams may have been caused by increased water temperature during summer and cold waves during winter leading to mortality, this has not been proved.

Proposed international collaborative studies in the future: Since China, Taiwan, Japan and Korea have active trade relationships and all these countries are affected by the Kuroshio Current, there is a high probability of transmission of pathogens between these countries. Therefore, there is a need to develop a joint monitoring program for pathogens of Manila clams that inhabit these areas.

Fig. 1. Major Manila clam production areas in South Korea

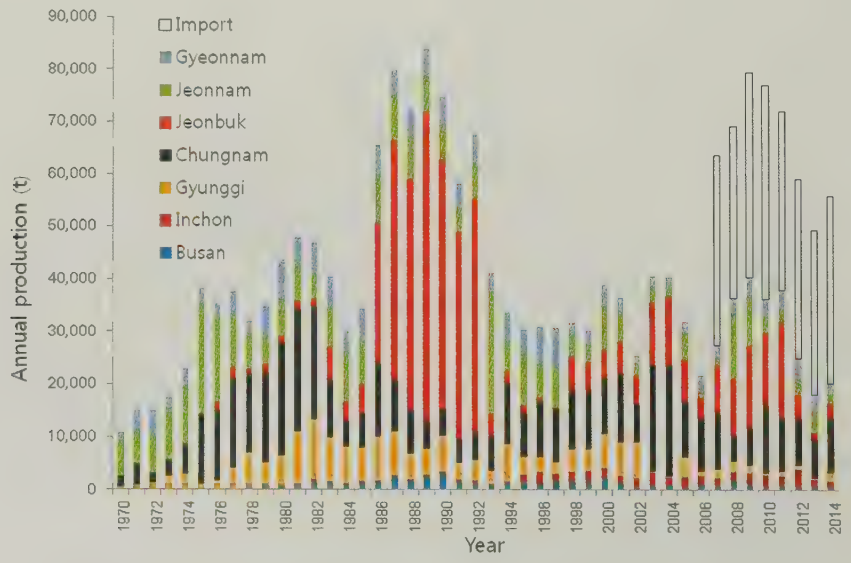

Fig. 2. Annual production and imported amount of Manila clam in South Korea.

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Country report from Portugal

Stefania CHIESA*1, Paula CHAINHO*2, and Francisco RUANO*3

General remarks on the Manila clam fishery

The Manila clam (*Ruditapes philippinarum*) is a bivalve species introduced into Portuguese estuarine environments since 1984. Originally from the Indo-Pacific region, it was introduced in Europe in the early 1970s in North European Atlantic and Mediterranean coastal waters for commercial cultivation; it was introduced in Northern Adriatic Sea in 1983 from an English hatchery. As for Portugal, it is believed that its introduction dates back to 1984, when it was reported at Ria Formosa. Since then the Manila clam was introduced in several different estuarine systems, such as the Tagus and Sado estuaries, Ria the Aveiro and Óbidos and Albufeira coastal lagoons.

Before Manila clam introduction, and until 2011, the clam production in Portugal was primarily based on two native species: *Venerupis corrugata* (Gmelin, 1791) and *Ruditapes decussatus* (Linnaeus, 1758). However, since the first appearing in FAO statistics in 2009, in the last 5 years the landing values of Manila clam improved consistently. Starting from 9 tons in 2009, the Portuguese production reached to more than 1000 in 2014. This phenomenon is particularly evident for the Tagus estuary (Trafaria and Costa da Caparica, Lisbon), where Manila clam has a wide distribution, densities of more than 5 ind/m^2 at some locations, and represents almost the entire clam production. Main declared landing sites also include Torreira (Ria de Aveiro), Sesimbra and Setúbal (Sado Estuary). The constant decline of native species populations determinated a change of the local fishing activities, as most commercial fishermen moved to more lucrative Manila clam catching.

The main issue concerning the Manila clam regards the high occurrence of illegal fishing, especially in the Tagus Estuary, conducted by unauthorized fishing devices, without permission and in interdicted production areas (heavy metal concentrations and microbiological contamination). Illegal harvesting of the Manila clam is most likely to represent more than 90% of the total catch.

A management plan for fishing of Manila clam, including the release of certified fishing concessions, is urgently needed in all productive sites.

Basic information on fishery

Geography and geomorphological characteristics: Manila clam fishing is mainly operated in sandy, muddy and oyster shell intertidal and subtidal areas (0 – 20 m) in estuarine coastal lagoons and estuaries.
Fishing method: Mechanical clam dredging, scuba diving and snorkeling, hand-raking, hand-operated tongs.
Fishery management: Commercial harvesting is limited to a maximum of 80 kg/day. No size limit or closure periods are regulated.
Standing stock assessment: Statistical survey of Manila clam fishing activity by DGRM (General direction of Natural resources, security and Maritime services) started in 2009, and currently a specific monitoring project is ongoing "2014 – Manila Clam – Current status, impact and fishing management in the Tagus estuary" funded by the Fisheries Program (PROMAR 31-03-01-FEP161)".

Basic information on aquaculture

No aquaculture based-production is actually

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occurring in Portugal.

Major constraints and countermeasures

The major constrain about Manila clam production is related to the illegal fishing, conducted by unauthorized collectors, using impacting fishing tools, in no-fishing zones. The absence of management plans for a sustainable exploitation and for clam traceability are the weak points in Portuguese market.

Habitat degradation: Although no specific studies have been conducted on the habitat degradation in the Tagus estuary, previous studies on areas where the Manila clam has been exploited indicated significant changes in the physical chemical characteristics of the water and sediments. Filter feeders are key elements in the control of primary production, water clarity and nutrient cycling. The action of dredges physically disrupts benthic substrate and may suspend sediment, increase turbidity, alter substrate composition, and cause sediment plumes. The re-suspension process of contaminated sediments (e.g. metals, tri-butyl tins, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and pesticides) will release contaminants into the surrounding water column that may then become biologically available. There are highly contaminated areas in the Tagus Estuary, which coincide with the area of occurrence of the Manila clam.

Overfishing: Over catching could be a risk as the production is improving, without a management regulation. Although harvesting has been intense along the last 5 years, the clam stock in the Tagus Estuary seems to be maintained with similar abundances.

Diseases and parasites: Occurrence of *Perkinsus olseni* has been reported for Portuguese populations of Manila clam, in the Tagus and Sado Estuaries, as in the Albufeira Lagoon. However, low frequencies have been observed, with no associated mortalities.

Climate change: No data are available.

Economic aspects: Mean price for kg ranges from 0.66 to 4.1 euros depending on time of the year and catching location. For example, in the Tagus Estuary is generally lower than 3 euro/kg.

Proposed international collaborative studies in the future: Patterns of genetic diversity for stock management and clam traceability; environmental quality and biomarkers assays for metal and organic contamination; management and fisheries/cultivation strategies.

Fig. 1. Main coastal lagoons and estuaries where *Ruditapes philippinarum* has been introduced and/or is currently exploited.

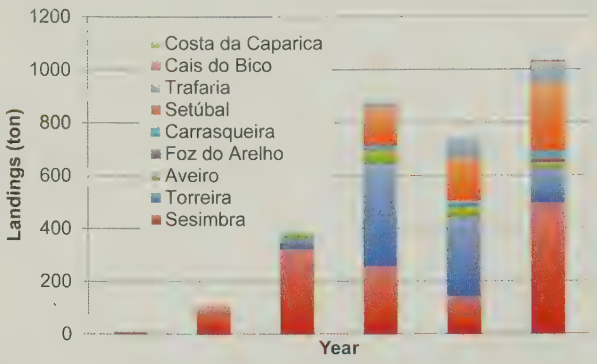

Fig. 3. Annual production of *Ruditapes philippinarum* in Portugal; sorted by main harbors.

Fig. 2. Manila clam densities in 2011 in the Tagus Estuary (ind/m^2). Original source: Garaulet, L.L. 2011. "Estabelecimento do bivalve exótico *Ruditapes philippinarum* (Adams & Reeve, 1850) no estuário do Tejo: caracterização da população actual e análise comparativa sobre a congénere nativa *Ruditapes decussatus* (Linnaeus, 1758) e macrofauna bentónica acompanhante". Tese de Mestrado em Ecologia Marinha, Faculdade de Ciências da Universidade de Lisboa, 77 p.

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Country report from Turkey

Serpil SERDAR*1

General remarks on the grooved carpet shell clam fishery

Shellfish production in Turkey has been based on fishery from wild stocks and also production/collecting areas are classified into 3 three categories (similar in European Union) where are called A, B and C according to amount of *Escherichia coli* in 100 g shellfish flesh and intravalvular liquid. Nowadays, there are totally 32 shellfish production areas in Turkey and all of them are including category B, it means that less than 4,600 *E. coli*/100 g shellfish flesh in 90% of samples. Before marketing, these shellfish must be depurated, heat-treated or relayed to meet Category A requirement and the rest 10% must not be excess 46,000 *E. coli* in 100 g shellfish flesh and intravalvular liquid. The grooved carpet shell clam, *Ruditapes decussatus*, is distributed along the all coastline of Turkey except from the Black Sea. Furthermore, it has been collected mainly from the Aegean Sea, especially from the Izmir Bay and there are three monitored areas in the Izmir Bay and one region in the Candarli Bay. This species is commercially important in Turkey due to foreign trade. The first statistical data about collecting of clam (*Ruditapes decussatus* and *Venus gallina*) was recorded as 158 t in 1986 (Fig. 1). This amount had been increasing until 1994 and it reached to 31 869 t. From 1995 to 1998, production of clam decreased to lowest value to 3550 t in 1998. Production amount began to increase again in 1999 (3585 t), after that it was fluctuated until 2006 (TURKSTAT, 2006). Additionally, *Ruditapes decussatus* and *Venus gallina* were firstly recorded separately in statistical reports in 2006 and amounts of *Ruditapes decussatus* and *Venus gallina* were recorded 1 266 t and 48,344 t, respectively (Fig. 2). In 2007 and 2008, total

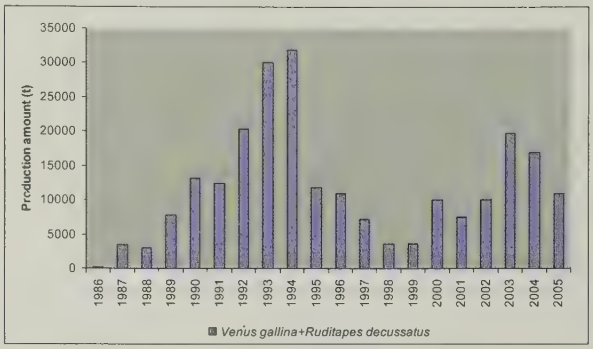

Fig. 1. *Venus gallina* and *Ruditapes decussatus* production in Turkey between 1986 and 2005.

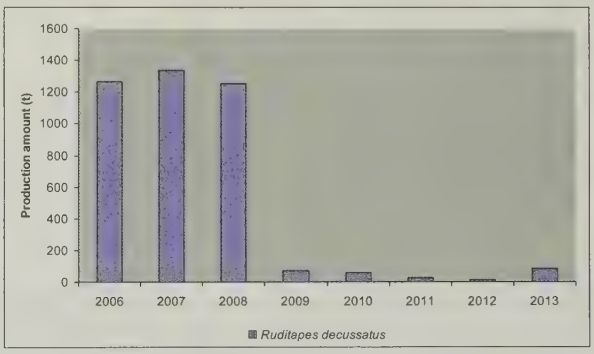

Fig. 2. *Ruditapes decussatus* production in Turkey from 2006 to 2013.

production of grooved carpet shell clam was the in similar with 2006. After 2008, this amount sharply reduced and it was collected 14.9 t in 2012. This value increased again in 2013 and production amount was determined 83.4 t (TURKSTAT, 2013). On the other hand, *Ruditapes decussatus* is native species along the Turkish coast but *Ruditapes philippinarum* is the foreign species and also it was firstly recorded in the both area which are Çanakkale (Dardanelles) Strait (2004) and in the Marmara Sea (2005) (Tuncer *et al.*, 2004; Albayrak, 2005).

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At the end of 2014, four regions were determined for producing Manila clam in the Marmara Sea by Directorate of Provincial, Republic of Turkey Ministry of Food, Agriculture and Livestock (Fig. 3). It will be commercially collected from these areas in the near future.

Basic information on fishery

Geography and geomorphological characteristics: *Ruditapes decussatus* is distributed generally sandy-muddy area along the Turkish coast and production area is between 1 m and 1.5 m depth.

Fishing method: Fishers usually dive with air supply from surface (operating with from boat) and/or they collect clam using with shovel and sieve.

Fishery management: Shell size regulations (sieve mesh size must not be smaller than 24 mm), fishing closed season (collecting of grooved carpet shell clam is prohibited from May 15 to September 15).

Standing stock assessment: Statistical survey by local fishers.

Basic information on aquaculture

Environmental characteristics: There has not been any aquaculture project for rearing grooved carpet shell clam but scientific studies approved that most of the lagoon areas in the Aegean Sea could be suitable for rearing of grooved carpet shell clam (Serdar and Lök 2007; Serdar *et al.*, 2007).

Culture methods: There is not any commercial size culture but scientific studies exhibited that net method could be used in the Izmir Bay for rearing clam seed especially using hard plastic net (Serdar *et al.*, 2007).

Others: As stated above, fishers usually collect clam from natural area by diving or using shovel-sieve, then they selected all clam by 24 mm sieve in land, remaining of clam under 24 mm are irregularly transferred and placed again in natural stock area for growing without project and controlled. This operation could be defined the hidden clam culture which are conducted for re-collection of clams by fishers and enhancement of natural stocks. On the other hand, scientific studies showed that reproductive activity (spawning stage) of grooved carpet shell clam is kept to continue throughout the year in the Izmir Bay where is very fruitful for growing natural population. But it needs fisheries management for sustainable production (Serdar and Lok, 2009; Serdar *et al.*, 2009; Serdar *et al.*, 2010).

Fig. 3. Production areas of *Ruditapes decussatus* (red color symbol), and planned production areas for *Ruditapes philippinarum* (green color symbol) in Turkey.

Major constraints and countermeasures

Habitat degradation: Clam population decreased dramatically due to coastal landscaping activities in 1990's. Change of bottom substrata and water quality parameters drastically effected production of this species.

Overfishing: Over and illegal collection could be caused to decrease clam population in Izmir Bay.

Diseases and parasites: To date, there is no report of any disease in clam from routine monitoring.

Climate change: Chlorophyll, total particulate matter and salinity values have changed especially in lagoon areas. Additionally, salinity increases during the summer due to lack of rain and over evaporation.

Others: *Ruditapes decussatus* more sensitive species and grow relatively slower than *Ruditapes philippinarum*. As stated before, *Ruditapes philippinarum* is not native species, and there is no record for distribution of *Ruditapes philippinarum* in the Izmir Bay until 2015. However, the main preoccupation point is the spreading out of this species to the Aegean Sea and Izmir Bay due to dominant than *Ruditapes decussatus*.

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